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MISDIVISION OF UNIVALENTS IN COMMON WHEAT.

By

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With 13 figures in the text.

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Introduction.

Isochromosomes were first reported in wheat by HÅKANSSON (1933), who called them "co-chromosomes." and by HUSKINS (1933), who referred to them as "chromosomes with two similar halves." Telocentric chromosomes were observed by HUSKINS and SPIER (1934) and LOVE (1938). Following the discovery by UPCOTT (1937), KOLLER (1938), and DARLINGTON (1939, 1940) that univalents tend to misdivide, it became obvious that both telocentrics and isochromosomes in wheat arise through misdivision. WINGE (1924) had, in fact, years previously figured misdividing univalents (his figures 24 and 25). LOVE described and figured misdivision in wheat in 1940, 1941, and 1943. SMITH, HUSKINS, and SANDER (1950) also reported misdivision of wheat univalents.

SEARS (1946) found a frequency of misdivision in microsporocytes of wheat (at anaphase I) which was sufficient to account for the frequency of telocentrics and isochromosomes observed in the progeny of monosomic plants. SÁNCHEZ-MONGE and MAC KEY (1948), however, reported that misdivision in their Swedish wheat material occurred mainly at the second meiotic division.

Materials.

The material studied consisted of monosomics in Chinese Spring wheat. Monosomics have the advantage over other sources of univalents (such as trisomics and hybrids) that every sporocyte has one univalent, and this one univalent is always the same chromosome. On the female side there is no elimination of the deficient gametes (SEARS, 1944), thus making possible the genetic determination of gametic ratios.

Monosome IX (the speltoid chromosome) was chosen for the study of misdivision for the following reasons: (1) It has been used in genetic and

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cytological studies much more extensively than any other wheat monosome. (2) It is distinctly heterobrachial (figure 2). (3) It is better marked genetically than any other wheat chromosome.

Chromosome IX of the variety Chinese Spring carries on its long arm the squarehead gene, *q*, for which dosages of from one to four are readily recognizable phenotypically (figure 1); the speltoid-suppressing

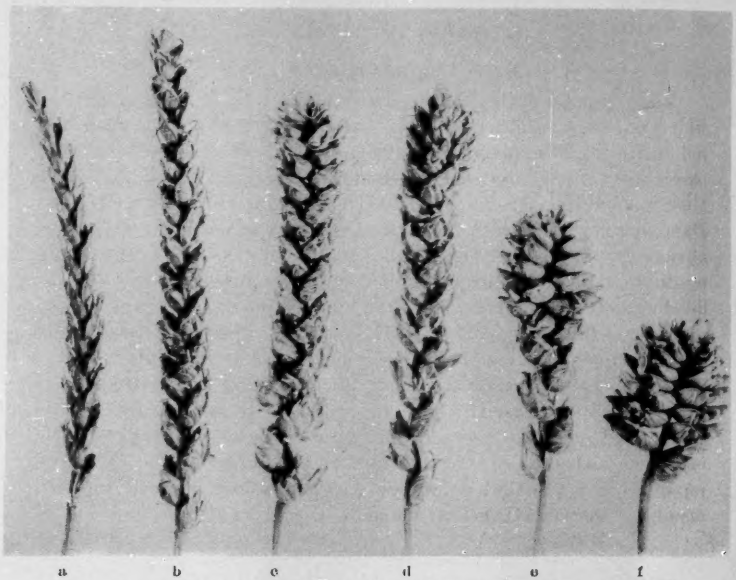

Fig. 1 a—f. Spikes of Chinese Spring wheat with various dosages of the long arm of chromosome IX. From left to right the dosages are 0, 1, 2, 3, 4. The spikes are from plants with (a) a nullisome, (b) a monosome for telocentric IX, (c) a monosome for iso-IX, (d) two normal IXs, (e) a telo and an iso, and (f) two isos. (0·80 ×.)

gene, *k*, located about 65 units away (PHILIPTSCHENKO, 1934; WATKINS and ELLERTON, 1940), and differentially effective at dosages of from zero to at least two; and a partially dominant gene for pubescent node approximately midway between the other two (GAINES and CARSTENS, 1926). The short arm is not only devoid of marker genes, but in this variety has little or no general effect on the plant, even in comparison with its complete absence (*cf. c* and *d* in figure 1).

In addition to mono-IX of Chinese Spring, monosome IXs were studied which had been transferred to Chinese from the wheat varieties Hope, Thatcher, and Red Egyptian. The transfers were made by cross-

ing the other varieties to Chinese mono-IX and then backcrossing for several generations to Chinese which was monosomic for an isochromosome IX. The Hope material was backcrossed five times. and Thatcher and Red Egyptian each four times.

Cytological Determination of the Frequency of Misdivision.

1. Misdivision of Monosome IX at TI.

The frequency of misdivision of univalent chromosome IX (table 1) was determined at T I from aceto-carmine preparations of microsporo-

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 2—4. T I in monosomic IX. Fig. 2. Normal division of univalent. (960 ×.) Fig. 3. Two arms going separately to upper pole. (960 ×.) Fig. 4. Long arm acentric on plate. (960 ×.)

cytes from mono-IX plants. Four plants were used in 1945 and two in 1949. Only those division figures were scored which were at late anaphase or telophase stages and which showed the univalent divided and its chromatids proceeding to the poles. Questionable figures were not included.

The univalent divided at the first division in 96% of 147 microsporocytes. Sánchez-Monge and Mac Key (1948) observed 97% of division at TI in their material.

The misdivisions observed may be grouped into three classes according to the number and kind of arms going to one pole, as follows: (*a*) One normal chromatid passes to one pole and the two arms of the other chromatid either pass separately to the other pole (figure 3) or one or both remain acentric on the plate (figure 4). Class *a* was not observed by Darlington (1939) in *Fritillaria* nor by Upcott (1937) in *Tulipa*,

but Darlington's type 4 is closely related to class *a*. (*b*) Two identical arms pass to one pole and the other two arms either go to the other pole (figure 5) or one or both remain acentric on the plate (figure 7). This class includes types 2 and 6 of Darlington (1939). (*c*) Three arms go to one pole and the fourth arm either passes to the other pole (figure 8) or remains on the plate. This class includes type 5 of Darlington. A fourth class, in which all four arms go to one pole, was not observed directly but can be inferred from TII observations (figure 12). Darlington's type 3, in which a single arm goes to each pole and two arms

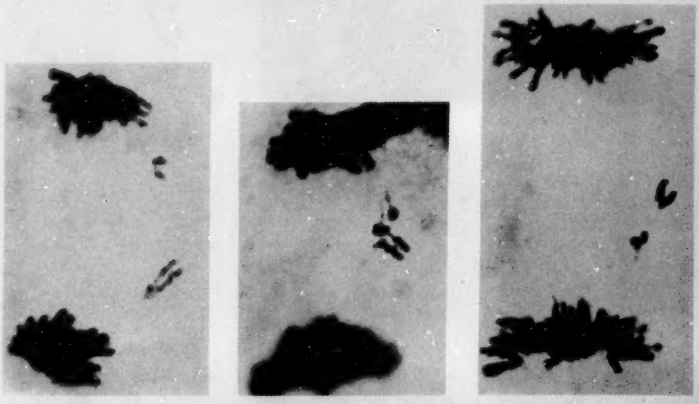

Fig. 5. Fig. 6. Fig. 7.

Fig. 5—7. T I in monosomic IX. Fig. 5. Isochromosome to each pole. (1080 ×.) Fig. 6. Two long arms to lower pole, two short to upper, each apparently with own centromere. (960 ×.) Fig. 7. Two long arms acentric on plate. (960 ×.)

are left on the plate, was not seen. His type 4, in which a long and a short arm go separately to each pole, was not observed either. One cell was found in which all four arms were evidently going separately to the poles (figure 6), but identical arms were going to the same pole. This figure, together with the observations of Li, Hsia, and Lee (1948) show that the centromere of a wheat univalent can divide at T I into four functional parts, as in *Tulipa* (Upcott, 1937).

Considering only the Chinese monosome IX, the most frequent kind of misdivision was class *b*. There were 29 misdivisions of this class out of 50 misdivisions seen.

In comparison with the variety of wheat used by Sánchez-Monge and Mac Key (1948), the Chinese Spring wheat showed a much higher frequency of misdivision. Sánchez-Monge and Mac Key found only 1·7% misdivision, as contrasted with a total frequency 39·7% in the

present material. DARLINGTON (1939), however, found 98% misdivision of univalents in *Fritillaria karadaghensis*.

With monosome IX of Chinese replaced by the homologous chromosome of any of three other varieties, the frequency of misdivision was lower (table 1). With the relatively small numbers of observations involved, differences among the three substituted lines were of doubtful significance. It is statistically demonstrable, however, that chromosome IX from Chinese is more susceptible to misdivision than is the corresponding chromosome from any of these other varieties. It must be noted, too, that even the substituted lines, with 13·7 to 23·6% misdivision, were much higher than the material of SÁNCHEZ-MONGE and MAC KEY. This suggests that the genome of Chinese, exclusive of chromosome IX, favors a high rate of misdivision of IX.

Fig. 8. T I in monosomic IX. Three arms to upper pole, one to lower. (1080 ×.)

There is an indication that with a lowered frequency of misdivision, class *c* misdivisions tend to become more frequent than class *b*. Whereas Chinese IX showed 29 in class *b* and only 17 in class *c*, Hope IX had only 3 in class *b* and 10 in class *c*, Thatcher IX had 3 in class *b* and 6 in class *c*, and Red

Table 1. *Frequency and types of misdivision of mono-IX at T I in four different strains.*

Class of misdivision	Distribution of chromosome arms at T I			Origin of chromosome IX, and number of microsporocytes in each class				
	Arms at one pole	Arms at other pole	Arms left on plate	Chinese		Hope	Thatcher	Red Egyptian
				1945	1949			
a	1 long, 1 short	1 long, 1 short	None	41	35	82	52	146
a	1 long, 1 short	1 long, 1 short (separ.)	None					3
a	1 long, 1 short	1 long	1 short	2			1	2
a	1 long, 1 short	1 short	1 long	2				1
a	1 long, 1 short	None	1 long, 1 short				1	
b	2 long	2 short	None	11	14	2	3	13
b	2 long	1 short	1 short		3	1		
b	2 short	None	2 long	1				1
c	1 long, 2 short	1 long	None	2	3	2	3	13
c	2 long, 1 short	1 short	None	5	7	8	3	11
c	2 long, 1 short	None	1 short					1
			Totals	64	62	95	63	191
			Percent misdivision	36·0	43·5	13·7	17·5	23·6

Egyptian IX had 14 in class *b* and 25 in class *c*. One outcome of a reduced frequency of class *b* compared with class *c* is a reduction in the ratio of isochromosomes to telocentrics, since class *b* misdivisions are responsible for most, or possibly all, of the isochromosomes produced.

2. Misdivision of Monosome IX at TII.

NISHIYAMA (1931) in oats, UPCOTT (1937) in *Tulipa*, KOLLER (1938) in *Pisum*, and DARLINGTON (1939) in *Fritillaria* reported misdivision of univalents at the second division, and SÁNCHEZ-MONGE and MAC KEY (1948) found 10·7% misdivision of 159 lagging univalent halves at TII.

Fig. 9. Fig. 10. Fig. 11.

Fig. 9—11. T II in monosomic IX. Fig. 9. Both halves of normally divided univalent in one T II cell. Right half misdividing. (960 ×.) **Fig. 10.** Sister T II cells, with misdivision in each. (960 ×.) **Fig. 11.** Sister T II cells, with misdivision at right, possibly at left. (1080 ×.)

Misdivision at TII consists of a pulling apart of the two arms at the centromere (figures 9—12). SÁNCHEZ-MONGE (1950, 1951) divides misdivisions into two classes: a-misdivisions (attraction-misdivisions), in which the two halves of the centromere separate early and lead the way to the poles; and p-misdivisions (push-misdivisions), in which the centromere is pulled apart only after the two arms have been carried toward opposite poles. In the present material all T I misdivisions would be classifiable as a-type; and SÁNCHEZ-MONGE's own figures of TI p-type misdivisions are not convincing. At TII, univalents may be pinched in two, as in SÁNCHEZ-MONGE's (1950) figure 2, but this type of misdivision is also probably less common than SÁNCHEZ-MONGE believes. At early TII stages the chromosome arms may be directed toward opposite poles, but at later stages the centromeres have almost always been found leading the way to the poles. To identify a p-type misdivision at as early a stage as in SÁNCHEZ-MONGE's (1951) figures 3b and 3d seems scarcely possible, if, indeed, one can even say for certain that these chromosomes would misdivide.

Typical TII misdivisions are shown in figures 10 and 11. Here the univalent evidently divided normally at the first division, and one com-

plete chromatid was included in each interkinetic nucleus. In the TII cell shown in figure 9 the univalent presumably was included entire in one TI nucleus. The two chromatids separated before the second division, and at TII they are behaving independently of each other. In figure 12 the laggards are behaving as two isochromosomes, with their positions suggesting that they have been joined at their centromeres during interkinesis. The univalent concerned can scarcely have passed undivided through the first division, for it should then have divided normally at TII without lagging. Presumably there was a misdivision

Fig. 12.

Fig. 13.

Fig. 12 and 13. T II in monosomic IX Fig. 12. Sister T II cells following probable 4:0 misdivision at T I. Centromere of each arm is at left. (960 ×.) Fig. 13. Both halves of normally divided univalent in one T II cell, neither misdividing. Lower chromosome, which is abnormally long and thin, was probably excluded from the T I daughter nuclei. (960 ×.)

at TI in which all four arms went to one pole and a naked centromere went to the other pole.

At TII, as at TI, Chinese mono-IX showed a much higher rate of misdivision than did the Swedish material. The data in table 2 show that 29·3% of the lagging chromosomes misdivided—nearly three times as many as found by SÁNCHEZ-MONGE and MAC KEY. Actually the rate

Table 2. *Second telophase behavior of monosome IX from various sources.*

Condition or behavior of mono-IX	Source of mono-IX and percent in each category				
	Chinese	Hope	Thatcher	Red Egyptian	Total
Not lagging	37·5	34·5	37·4	41·2	37·8
Passing entire	4·9	4·2	2·4	3·3	4·1
Misdivided	18·3	10·9	21·7	17·0	17·1
Lagging, unclassifiable	26·5	45·4	33·8	28·8	31·2
Abnormal	4·9	4·2	2·4	3·3	4·1
Telocentric	7·9	0·8	2·4	6·5	5·7
No. cells examined	328	119	83	153	683

of TII misdivision for Chinese may be somewhat higher than 29·3%, for the "lagging, unclassifiable" class included some early telophases in which the laggard was still subject to misdivision. Also, the total used in calculating the 29·3% included the telophases with a lagging telocentric, although telocentrics are not subject to TII misdivision. Likewise, the "abnormal" II telophases were included, and these had a long, thin laggard, as in figure 13, which presumably had been excluded at TI and was not participating in the second division.

It is clear from observations of late TII that many of the second-division laggards fail to be included in the telophase nuclei. They form micronuclei which can conveniently be counted at the quartet stage. Data are given in table 3 for micronuclei in quartets of Chinese mono-IX. Calculations from the data in table 2 show that there would be 1·62 micronuclei per quartet if none of the "lagging, unclassifiable" chromosomes divided and none of the laggards were included in the quartet nuclei; whereas 1·21 micronuclei per quartet were actually observed. This suggests that about 25% of T II laggards are included in the quartet nuclei. Division of a few of the "lagging, unclassifiable" chromosomes would raise this value slightly. The data of SÁNCHEZ-MONGE and MAC KEY (1946) indicate about the same frequency of inclusion of TII laggards.

Table 3. *Distribution of micronuclei in quartets from Chinese monosomic-IX.*

Micronuclei in each microspore	Quartets of each type %
0–0–0–0	29·3
0–0–0–1	31·7
0–0–1–1	27·4
0–1–1–1	7·3
1–1–1–1	1·2
0–0–0–2	1·5
0–0–1–2	0·9
0–1–1–2	0·6
Total No. quartets	328

In table 2 it will be noted that monosome IX derived from the varieties Hope, Thatcher, and Red Egyptian showed essentially the same behavior at TII as did IX from Chinese. Only Hope IX seemed to differ substantially in any category, and the differences here may not have been significant.

Counts of micronuclei in the Hope-IX and Red-Egyptian-IX material showed only 0·94 and 0·91, respectively, per quartet. This indicates inclusion of a larger fraction of the TII laggards than in Chinese mono-IX, with the probable effect of increasing the number of telocentrics. However, selective elimination of the telocentrics formed is a possibility.

3. Misdivision of Other Wheat Monosomes.

The occurrence of such high rates of misdivision in this material raises the question whether other Chinese chromosomes, when univalent,

also undergo frequent misdivision at TI and TII. Evidence from both genetic and cytological sources indicates that they do frequently misdivide. From all of the 19 other monosomics available, plants with a telocentric or an isochromosome have been obtained, sometimes in rather high frequency. Only four rather small populations, all from self-pollination, have been analyzed completely, but the results may be taken as having some significance when considered altogether. Among seven chromosome IV's transmitted through the egg, one was a telocentric; among six of chromosome VI, none was a telocentric; among 12 of XI, one was an isochromosome; and among nine of XX, one was telo and one iso. Thus there were four telos and isos among 34 chromosomes

Table 4. *Frequency of misdivision of various chromosomes in Chinese Spring Wheat.*

Chromosome	Variety from which derived	Misdivision at T I		Misdivision at T II	
		%	Total No. cells	%	Total No. cells
II	Red Egyptian	11·4	44	27·6	58
V	Chinese	26·7	15	—	—
XI	Hope	29·4[1]	17	43·7	16
XVIII	Hope	—	—	25·0	12

[1] A minimum value, for 2:2 misdivisions of this approximately isobrachial chromosome could not be distinguished from normal divisions.

transmitted through the egg. In addition there were three telos in these families (one each of IV, VI, and XX) presumably transmitted through the pollen, since they were present as monosomes.

Cytological study of a few chromosomes (table 4) has shown misdivision rates comparable with those found for IX. Some of the figures are small, but the evidence is unmistakable that other chromosomes than IX are subject to frequent misdivision in Chinese wheat.

4. Frequencies of Telocentrics and Isochromosomes Expected.

SÁNCHEZ-MONGE and MAC KEY, in trying to explain the origin of isochromosomes in their material, where no class *b* misdivisions were observed at TI, suggested an origin of isos through non-disjunction of the two chromatids of a telocentric at the pollen mitosis. This suggestion was based on RHOADES' (1938, 1940) discovery of such an origin for an isochromosome in maize, and on DARLINGTON's (1940) observation of non-disjunction of telocentrics at the pollen mitosis in *Fritillaria*. There is little to support the suggestion as it applies to wheat. Isochromosomes in wheat are predominantly of maternal origin. Also, in the Chinese mono-IX material, no instances of non-disjunction at the pollen mitosis could be found, although telocentrics must

have been present in considerable frequency. Furthermore, passage of both arms of the telocentric to the generative nucleus would leave the vegetative nucleus completely deficient for chromosome IX. Such deficient pollen functions only rarely in competition with normal pollen. It seems likely that isochromosomes were produced at TI in SÁNCHEZ-MONGE and MAC KEY's material, but in such low frequency as to escape observation. SMITH, HUSKINS, and SANDER (1950) believed that isochromosomes in their wheat material arose through misdivision at TI.

According to table 1, misdivision occurs in 39·7% of first divisions in Chinese mono-IX. Over half of these misdivisions are of a type which results in production of isochromosomes. Clearly, however, misdivision at TII must result in the loss of some of these isochromosomes. Over 60% of univalent halves were observed to lag at TII, and about 30% of the laggards misdivided to form telocentric chromosomes. There is some evidence from the work of SMITH, HUSKINS, and SANDER (1950) and SÁNCHEZ-MONGE (1951) that isochromosomes have less tendency than normal chromosomes to lag (and hence misdivide) at TII, but misdivision of isochromosomes at TII has been observed several times (e. g., figure 12). DARLINGTON (1939) reported that isochromosomes produced at TI in *Fritillaria karadaghensis* always underwent misdivision into two telocentrics at TII.

From the TI data (table 1) there can be calculated the approximate number of isochromosomes and telocentrics produced at the first division. In the total of 126 microsporocytes observed at TI, there were 25 in which the two long arms went to one pole and the two short arms to the other pole. In addition, there were 4 cells in which two identical arms went to one pole, and one or both of the other two arms were left on the plate. Where three arms went to one pole (17 cells), there is no evidence as to how disjunction occurred at the next division. It is perhaps fair, however, to credit one-third of these with giving rise to an isochromosome. Thus from the 126 microsporocytes, one may take for a maximum the production of $2 \times 25 + 4 + 17/3 = 59{\cdot}7$ isochromosomes. The normal chromosomes totalled $2 \times 76 + 4 + 2 \times 17/3 = 167{\cdot}3$, and the telos were $4 + 3 + 2 \times 17 = 41$. At the end of the first division, then, 22·3% of the chromosomes would be isochromosomes, assuming no greater tendency for chromosome loss following misdivision than following normal division, and 15·3% would be telocentrics.

At TII, it seems safe to predict, misdivision would reduce the number of isochromosomes and increase the number of telocentrics sufficiently that the latter would exceed the former. To make a more precise prediction is probably unwarranted, because of such uncertainties as possible selective elimination of telos and isos at TI and TII. UPCOTT (1937) reported elimination of all the telos produced at TII in *Tulipa*.

Genetic Determination of the Frequency of Misdivision.

The frequencies of isochromosomes and telocentrics in gametes produced by monosomic IX plants were determined by pollinating mono-IX with normal pollen and observing the constitution of the resulting plants with respect to chromosome IX. The constitution of the eggs was deduced by subtracting one normal, paternal chromosome IX from the complement of each of the plants. The data are presented in table 5.

Plants with three doses of the long arm of IX, as determined genetically mainly by dosage of the squarehead gene, were assumed to have received through the egg an isochromosome for the long arm. Two normal chromosomes could theoretically have been contributed by the female parent, but transmission of this sort is rare in wheat. Also, several of the triplex individuals were examined cytologically, and all had an isochromosome. One plant had a simplex sector, presumably as the result of somatic loss of the isochromosome. Included in the triplex group were four plants which were actually quadriplex, but which were assumed to have been triplex at the zygotic stage (SEARS, 1952). Three of these had a normal IX, an isochromosome, and a telocentric. The telocentric presumably arose somatically through misdivision of the isochromosome. The fourth quadriplex plant had two normal IXs and an isochromosome. The isochromosome was assumed to have originated through somatic misdivision of a telocentric.

Duplex plants had either two normal chromosomes or a normal and a long-arm telocentric—a distinction which had to be made cytologically. The contribution of the egg was of course in the first case a normal chromosome and in the second a telocentric. One or possibly two of the plants with a telocentric chromosome had evidently lost the telocentric in a sector.

Simplex individuals received no long arm of IX through the egg. They may have received a telocentric or an isochromosome for the short arm.

Table 5. *Frequency of telocentrics and isochromosomes involving the long arm of chromosome IX in female gametes produced by mono-IX plants.*

Season	Number of eggs fertilized	Number plants surviving to maturity	Chromosome IX or derivatives transmitted to		Normal chromosome		Telocentric		Isochromosomes		Normal plus telocentric	
			No.	%	No.	% (of those transmitted)	No.	%	No.	%	No.	%
1944	105	105	12	11·4	9	75·0	2	16·7	1	8·3	0	0·0
1945	717	680	145	21·3	120	82·8	14	9·6	10	6·9	1	0·7
Total	822	785	157	20·0	129	82·2	16	10·2	11	7·0	1	0·6

According to table 5, 28 (17·8%) of the 157 gametes receiving a chromosome IX or derivative had a telocentric or an isochromosome for the long arm of IX. In addition it may reasonably be assumed that substantially the same number of eggs received isochromosomes or telocentrics involving the short arm of chromosome IX. Only one of these short arm derivatives would have been detected, since all but the egg with both a normal chromosome and a telocentric would have produced plants falling into the simplex class, members of which were not examined cytologically. Correcting, then, for these short-arm types, one may add 27 to the total number of isochromosomes and telocentrics and to the number of chromosome IXs or derivatives transmitted. The totals now become 184 (23·4%) eggs receiving a chromosome IX or derivative, of which eggs 33 (17·9%) received a telocentric and 22 (12%) received an isochromosome for one arm or the other of chromosome IX.

Thus it appears that at least 29·9% of the chromosome IXs transmitted were isochromosomes or telocentrics. The actual frequency may have been somewhat higher than this, for, as pointed out in the following paper (SEARS, 1952), isochromosomes and particularly telocentrics are sometimes lost in somatic tissue, and this suggests that some of the plants classified as receiving no chromosome IX or derivative through the egg may actually have received a telocentric or isochromosome and lost it at an early stage of development.

The relative frequency of isochromosomes and telocentrics obtained (12% vs. 17·9%) is in agreement with that expected from the cytological studies (22·3% vs. 15·3% at TI, with a decrease in isos and an increase in telos at TII). However, the total of 29·9% for isos and telos actually transmitted is less than even the 37·6% predicted from TI data before the expected increase at TII from addition to the number of telos and reduction in the number of normal chromosomes (and isos). One explanation for this discrepancy would be a greater tendency for loss of misdivided chromosomes, particularly loss of telocentrics at TII.

A genetic check was undertaken of the reduced rate of misdivision of the monosome IX which had been introduced from the variety Hope. Selfed populations of 394 and 400, respectively, were grown from the same plants of Chinese mono-IX and Hope mono-IX as had been used for the cytological studies. The entire 400 of the latter were scored, but only 212 of the former, which were about two weeks later in maturity. Presumably very few, if any, triplex and quadriplex plants were missed, however, for these tend to be earlier than the monosomics and nullisomics. The results are given in table 6. It will be seen that there were only four plants from Hope-IX with as many as three doses of the long arm of IX, whereas from Chinese-IX there were at least ten such plants. The difference is not statistically significant, but it is in substantial cor-

respondence with the difference observed cytologically in frequency of misdivision at TI, which was 13·7% against 39·7%.

Genetic data based on larger numbers are available in the literature for the frequency with which isochromosomes arise from monosomes. These data are for selfed monosomics, but it may be assumed that all the isochromosomes were transmitted through the egg, and that the functioning pollen all carried a normal chromosome IX except for a small fraction, calculable from the number of nullisomics, which were deficient for IX. NILSSON-EHLE's data (1920, table 1; 1921, tables 4 to 9), taken from 10,477 plants, show 3% of iso-IX among chromosome IXs maternally transmitted. LINDHARD (1922, tables 1 to 5, 8 to 10), in a study based on 38,237 plants, found 2·9% isochromosomes. UCHIKAWA (1941, table 5) reported 4·1% isochromosomes among those maternally transmitted to 2677 offspring. ÅKERMAN and MAC KEY (1948) report frequencies amounting to 1%, 1·1%, and 4·8%, respectively, in three different lines, in which 6631, 29,976, and 1265 plants, respectively, were grown. SMITH, HUSKINS, and SANDER (1949) found 0·3% isochromosomes among chromosomes transmitted maternally to 2084 offspring. These frequencies of 0·3% to 4·8% average less than half the 7% reported in table 3.

Table 6. *Classification of offspring from mono-IX material of different origin.*

Doses of the long arm of IX	Source of chromosome IX, and number of plants in each class	
	Chinese	Hope
0	13	13
1	171	333
2	18	50
3	7	4
4	3	0

Female transmission of chromosome IX and its derivatives in the experiment summarized in table 3 (20%) was similar to that observed by NILSSON-EHLE (23%), LINDHARD (17%), UCHIKAWA (18%), ÅKERMAN and MACKEY (11%, 19%, and 13%), and SMITH, HUSKINS and SANDER (13·3%).

Discussion.

The manner of origin of isochromosomes and telocentrics in wheat now seems fairly clear. At the first division of meiosis, univalents undergo a variety of types of misdivision, and both telocentrics and isochromosomes are produced. At the second division, some of the isochromosomes are converted to telocentrics by a second misdivision, and additional telocentrics are formed by misdivision of some of the chromosomes which divided normally at the first division. There is a strong tendency, however, for telocentrics formed at the second division to be lost.

Direct observations of first and second division misdivision have been made on microsporocytes, whereas practically all of the isochromosomes and telocentrics recovered are formed during megasporogenesis.

It is thus possible that isochromosomes formed at the first division fail to persist as such, and that isochromosomes actually transmitted are produced by misdivision of telocentrics at some post-meiotic division. Since the observations on microsporogenesis provide a satisfactory explanation of the origin of telocentrics and isochromosomes, however, there seems to be little reason for assuming that anything substantially different happens at megasporogenesis.

SÁNCHEZ-MONGE and MAC KEY (1948) believe that chromosomes left out of the daughter nuclei at TI form micronuclei which later reform into chromosomes and participate normally in the second division. In the present study, some abnormally long chromosomes were found at TII (figure 13) which were clearly not under the influence of the spindle. These (listed as abnormal in table 2) are best interpreted as chromosomes which failed to be included in either daughter nucleus at TI. Although doubtful cases were excluded from this class, it seems scarcely possible that there were enough of these abnormal chromosomes to account for all the laggards excluded at TI. The 4·9% recorded in table 2 would be accounted for by only 0·098 micronuclei per interkinetic cell, whereas examination of interkinesis showed 0·32 micronuclei per cell, and SÁNCHEZ-MONGE and MACKEY found an even larger number in their material (1·05 per cell). Evidently some of the chromosomes excluded at TI either are included in one of the TII groups or participate in the second division as laggards.

The evidence is fairly conclusive that isochromosomes in wheat are produced mostly or entirely at the first division. DARLINGTON (1940) found that isochromosomes in *Fritillaria* may arise at the pollen mitosis through misdivision of telocentrics; but it is not known whether these isochromosomes are actually transmitted. RHOADES (1940) has shown that in maize where the telocentric is supernumerary, a pollen grain in which an isochromosome has been produced at the pollen mitosis and has gone to the generative nucleus has a competitive advantage over other grains carrying the telocentric, because the vegetative nucleus of this grain has a normal chromosome complement. Thus the way in which isochromosomes originate in a particular species depends upon the frequency with which isos are produced at TI, their ability to pass unchanged through the second division, the frequency with which telos give rise to isos at post-meiotic stages, and whether or not pollen competition may favor gametes carrying isos produced at a particular division.

Summary.

In the wheat variety Chinese Spring, misdivision of monosome IX was observed at TI in 39·7% of microsporocytes and at TII in at least 29·3% of cells with a lagging univalent. Mono-IX from three other

varieties, but with a Chinese Spring background, also showed high frequencies of misdivision, but less at TI than the Chinese mono-IX. Other Chinese monosomes likewise misdivided frequently.

Misdivision at TI resulted in the production of both telocentrics and isochromosomes. Misdivision at TII involved some of the newly produced isochromosomes as well as normal chromosomes, but there was a high frequency of loss of TII laggards. No tendency for telocentrics to misdivide and form isochromosomes was observed at the first microspore mitosis.

Genetic tests showed that in 17·8% of eggs which transmitted a chromosome IX or derivative it was an isochromosome or telocentric for the long arm which was transmitted. Since an equivalent frequency of short-arm derivatives were presumably transmitted also, there is substantial agreement with the cytological observations, if it be assumed that most of the telocentrics produced at TII are lost.

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THE BEHAVIOR OF ISOCHROMOSOMES AND TELOCENTRICS IN WHEAT.

By

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With 4 figures in the text.

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Isochromosomes and telocentrics, which commonly result from misdivision of univalent chromosomes, have now been observed in a variety of plants. Where detailed studies have been made, certain types of aberrant behavior have usually been noted.

Rhoades (1938, 1940) found a telocentric in maize to be unstable in somatic tissue, tending to become reduced in size or to be entirely lost. At the pollen mitosis it occasionally gave rise to an isochromosome. No somatic aberrations of the isochromosomes were observed. Darlington and Janaki-Ammal (1945) found that bivalents and quadrivalents formed from isochromosomes in *Nicandra* tend to lag at the first meiotic division, and that the isos also lag at mitosis.

Aberrant behavior of the supernumerary, nearly inert B-type chromosomes of maize has been reported, consisting chiefly of a tendency to undergo diminution in size (Randolph, 1941) and to suffer non-disjunction at the second microspore mitosis (Roman, 1947). Some B chromosomes are evidently telocentric (McClintock, 1933; Roman, 1947), but since others which also behave aberrantly have two arms (Randolph, 1941; Darlington and Upcott, 1941), it is doubtful that the aberrant behavior of any of the B's can be attributed to the terminal position of the centromere. Furthermore, Müntzing (1944, 1945, 1946) has reported a supernumerary, bibrachial chromosome in rye which also undergoes almost regular post-meiotic non-disjunction.

Wheat (*Triticum aestivum* L. *emend.* Fiori et Paoletti) has been the subject for several studies on isochromosomes and telocentrics. Love (1943) noted that various isos and telos in wheat commonly misdivide at anaphase I when they are unpaired. Other published work with wheat has dealt largely or entirely with derivatives of the speltoid chromosome,

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which has been referred to as the C chromosome by Winge (1924) and others, and which is chromosome IX in Sears' (1944) series. Sears (1946) found no striking differences in the female transmission and misdivision frequencies of univalent iso- and telo-IX in comparison with normal IX, but iso-IX and particularly telo-IX were unstable somatically. Li, Hsia, and Lee (1948) observed meiotic misdivision of both iso- and telo-IX. Smith, Huskins, and Sander (1950) reported behavior of univalent iso-IX similar to that of normal IX, but with perhaps slightly less tendency of the isochromosome to be excluded at TII. Sánchez-Monge (1951) noted a similar tendency for more frequent inclusion of the iso at TII.

In the present study, isochromosomes and telocentrics for the long arm of chromosome IX of Chinese Spring wheat were used. The formation of these chromosomes through misdivision of mono-IX has already been described (Sears, 1952).

Telocentric chromosomes usually go through the meiotic divisions in normal fashion when present as a pair or when accompanied by a normal homologue. Occasionally the telocentric and its homologue are found unpaired at MI, a situation ascribable to the decreased likelihood of chiasma formation in the relatively short telocentric.

The amount of pairing of the isochromosome for the long arm of chromosome IX [Huskins' (1946) Cil] with normal IX (or with a homologous isochromosome) is quite variable, but apparently never exceeds 50%. Håkansson (1933) found pairing in up to 49% of pollen mother cells, Åkerman and Mac Key (1948) in 3%, and Smith, Huskins, and Sander (1950) in 48%. The latter authors found 38% pairing of two isochromosomes. When the isochromosome does not pair with its homologue, it usually pairs with itself to form a ring univalent. When it does pair with another chromosome, it apparently behaves normally throughout meiosis.

Frequency of Misdivision.

The frequency with which telocentrics and isochromosomes misdivide when unpaired can be determined genetically for comparison with the frequency for normal chromosome IX already determined (Sears, 1952). With telocentrics only one type of misdivision is detectable — that which forms an isochromosome. Presumably the rate with which isochromosomes are formed from telocentrics should be directly comparable to the rate at which isochromosomes for the same arm are formed from normal chromosomes. From misdivision of isochromosomes, twice as many telocentrics for the arm concerned are formed as from an arm of a normal chromosome undergoing the same amount of misdivision. New isochromosomes are also formed from misdivision of an isochromosome, but the new isos are indistinguishable from the original one.

Determination of the frequency of misdivision of unpaired telocentrics and isochromosomes for the long arm of chromosome IX was made genetically by pollinating iso- and telomonosomics by plants of normal constitution and observing the offspring for dosage of squarehead and other genes. Plants with a telocentric monosome gave only simplex and duplex offspring except where the telocentric had been converted into an isochromosome; and plants with an isomonosome gave only simplex and triplex offspring except where the isochromosome had given rise to a telocentric. The data are presented in table 1, along with data for normal IX for comparison (from SEARS, 1952).

Table 1. *Frequency of telocentrics and isochromosomes in female gametes produced by plants monosomic for a normal chromosome IX or for a telocentric or an isochromosome involving the long arm of chromosome IX.*

Type of monosome	No. gametes analyzed	Monosome or derivatives transmitted to		Gametes with telo.		Gametes with iso.	
		No.	Percent	No.	Percent (of chromosomes transmitted)	No.	Percent (of chromosomes transmitted)
Telo	769	83	10,8	72	86,7	11	13,3
Iso	358	50	14,0	7	14,0	43	86,0
Normal	785	157	20,0	17	10,8	11	7,0

Telocentric IX yielded 13.3 $\pm$ 3.7% isochromosomes as against 7.0 $\pm$ 2.0% for normal IX. Although the difference is not statistically significant, the data do not exclude the possibility of a considerably higher rate of misdivision of the telocentric. Iso-IX, on the other hand, which was expected to yield twice as many telocentrics as normal IX, gave only 14.0 $\pm$ 4.9% compared with 10.8 $\pm$ 2.4% for normal IX. Here again the difference from expectation is not statistically significant, but larger numbers might show less frequent misdivision of the isochromosome than of the normal.

The frequency of misdivision of iso-IX at TI was determined cytologically by LI, HSIA, and LEE (1948) to be 6.48%. This value is lower than obtained by SEARS (1952) for normal IX, but environmental effects cannot be ruled out as the cause of part or all of the difference. The frequency of misdivision of the iso at TII in the material of LI, HSIA, and LEE can scarcely be calculated from the data they give because some of the univalents they attempted to score were evidently still on the plate and not yet properly classifiable as divided or misdivided (e.g., their figure 12 right and figure 14 left). Also they interpreted certain TII misdivisions, as in their figure 14 right, as due to misdivision at TI.

LI, HSIA, and LEE (1948) reported misdivision of telo-IX to form iso-IX at TII but not at TI. Inspection of their drawings, however,

leads to the conclusion that what they interpret as TII misdivision is actually normal distribution of an isochromosome formed at TI, and that their supposedly normal TII division is actually misdivision of an isochromosome formed at TI. Thus, lumping together their TI and TII data, one finds a frequency of 12% misdivision at TI, somewhat higher than for their isochromosome, but not significantly so.

The telocentrics used in determination of misdivision frequency numbered 16 of different origin—all having arisen from the same isochromosome. No significant differences were apparent among these in frequency of misdivision, but numbers of isochromosomes produced amounted to no more than two for any one telocentric. Further tests of the four telocentrics producing the greatest frequency of isochromosomes showed only 1.6% isochromosomes among 980 offspring with none of the four especially high; while three telocentrics which had yielded no isochromosomes gave 1.3% among 787 offspring—substantially the same frequency.

The transmission of telo-IX and iso-IX appears at first to be lower than that of normal-IX, the values being 10.8% and 14.0% as against 20%. It must be noted, however, that the data for telo-IX and iso-IX were obtained in 1944, the same season in which normal IX showed only 11.4% transmission (SEARS, 1952). In another season iso-IX showed over 25% transmission.

One chromosome included among the 11 isochromosomes produced from telocentrics was actually a heterobrachial chromosome with one arm apparently slightly shorter than the long arm of IX and the other much shorter. Also present was a very short centric fragment of IX. Pairing was observed between the two arms of the larger chromosome. Since the spikes of the plant coming from this gamete apparently had only one dose of the squarehead gene, at least two doses of the speltoid suppressor, and just two doses of the pubescent-node gene, it seems probable that the larger chromosome had one arm which was normal except for loss of the region carrying the squarehead gene, and one arm carrying none of the identifiable genes. The fragment presumably carried none of these genes. The plant appeared to be of uniform constitution, suggesting that the aberrant chromosomes may have been present in the gamete. They may equally well have been formed, however, during megagametophyte development or in an early embryonic stage.

Transmission.

On the male side, intensive selection occurs against gametes deficient for the long arm of chromosome IX. Thus, only 1.5% of offspring of mono-IX selfed were nullisomic (SEARS, 1944), indicating that only

about 2% of the functioning male gametes were deficient for chromosome IX. Results of NILSSON-EHLE (1920, 1921), UCHIKAWA (1941), ÅKERMAN and MAC KEY (1948), and SMITH, HUSKINS, and SANDER (1949) for much larger numbers are comparable, their results showing 0.67%, 1.35%, 0.99%, and 0.62%, respectively, of nullisomic IX obtained from mono-IX selfed.

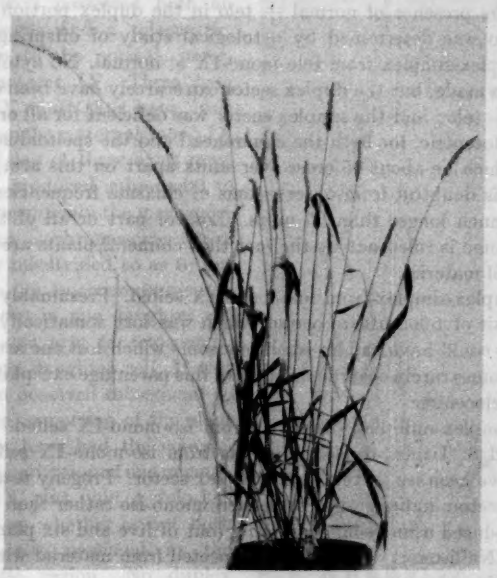

Fig. 1. Chimera in *Triticum aestivum* var. Chinese Spring. Spike on the left has lost the long-arm telo-IX present in the rest of the plant. 0.09 ×.

From mono-telo-IX, 3.0% of 439 offspring were nullisomic, indicating little, if any, difference in competitive ability between pollen carrying telo-IX and that carrying normal IX.

The isochromosome for the long arm of IX is less favorable to pollen than is the telocentric. Of 197 successful male gametes analyzed from a plant with an isomonosome, 26.4% were nullisomic, only 43.6% had the isochromosome, and the remaining 30.0% possessed telocentrics presumably derived from the isochromosome by misdivision. Since the yield of telocentrics from iso-IX has been shown to be only about 14% of the transmitted chromosomes, selection in favor of the telocentrics must be assumed here to account for their high frequency (40.7% of the transmitted chromosomes).

Behavior in Somatic Tissue.

Eight instances interpreted to be complete somatic loss of telo-IX have been observed, as follows:

1 to 3. Chimeral plants duplex-simplex for the squarehead and speltoid-suppressing genes, from mono-IX × normal. The monosomic nature of the simplex sectors of two of these plants was verified cytologically. The presence of normal + telo in the duplex portion of one of these two was determined by cytological study of offspring.

4. Duplex-simplex from telo-mono-IX × normal. No cytological analysis was made, but the duplex sector can scarcely have been other than normal + telo; and the simplex sector was deficient for all or nearly all of the telocentric, for both the squarehead and the speltoid-suppressing genes, which lie about 65 cross-over units apart on this arm, had been lost. It is doubtful from observations of chiasma frequencies that this arm is much longer than 65 units. Loss of part or all of the normal chromosome is ruled out by the fact that chimeral plants are not found in control material.

5. Duplex-simplex from telo-mono-IX selfed. Presumably this plant had a pair of telocentrics, one of which was lost somatically. Alternatively, it could have had an isochromosome which lost one arm, but isochromosomes rarely occur in cultures of this parentage except in company with a telocentric.

6. Simplex-nulliplex (figure 1) from iso-mono-IX selfed.

7 and 8. Triplex-duplex chimeras from iso-mono-IX selfed. These must have been iso + telo in the triplex sector. Progeny tests from the duplex sectors indicate that these were mono-iso rather than di-telo, for each produced a nullisomic offspring (out of five and six plants, respectively). Nullisomics would not be expected from material with a pair of telocentrics.

The somatic loss of a telocentric chromosome III has also been observed (figure 2).

The post-meiotic conversion of a telocentric into an isochromosome evidently occurred in one plant mentioned in the companion paper (SEARS, 1952). This plant had an iso-IX and two normal-IXs, of which the iso-IX and one normal IX must have come from the female parent. Since this parent was simple mono-IX, however, both an iso-IX and a normal IX could not have been present as such in the megaspore, for this would have involved obtaining three long arms of IX from a single chromosome. Therefore, the megaspore must have carried a normal IX and a telo-IX, the latter subsequently undergoing misdivision to produce an iso-IX. This misdivision may have taken place either during gametophyte formation or early enough in sporophyte development that a single cell gave rise to the initials of all the spikes. RHOADES (1940)

observed no production of isochromosomes from telocentrics in sporophytic tissue, but found it occurring occasionally at the pollen mitosis.

Although RHOADES (1940) observed no somatic aberrations of his isochromosome in maize, there is little doubt that these occur in wheat. Three of the four quadriplex plants mentioned in the companion paper (SEARS, 1952) possessed both an isochromosome and a telocentric, in addition to the normal, paternal chromosome IX. Three long arms of IX must thus have been derived from the maternal, mono-IX plant, and one of these arms must have been produced subsequent to meiosis. Presumably the megaspore received an isochromosome which eventually misdivided so as to put three arms (an isochromosome and a telocentric) in one daughter cell and only one arm (a telocentric) in the other daughter cell. If this misdivision occurred subsequent to fertilization, a portion of the plant might then have had the complementary constitution of one normal, paternal IX plus only a telo-IX, depending on whether or not both daughter cells persisted in the meristematic region. No duplex (or triplex) sectors were observed in the three plants mentioned, but in a plant of identical constitution from another population (table 1, line 1), a triplex sector, with only an iso-IX and a normal IX, was found, suggesting that here one of the two daughter cells resulting from misdivision and at least one cell of the parental type persisted in the meristematic region.

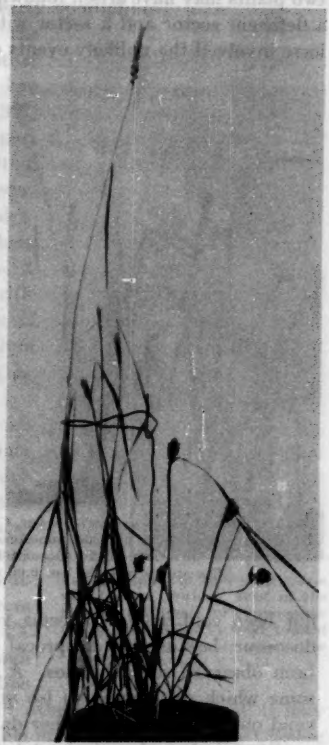

Fig. 2. Chimera in *Triticum aestivum* var. Chinese Spring. Spike on the left has a telocentric chromosome III which is lacking in the rest of the plant. 0.13 ×.

Two plants have been found which seem best explained as involving the somatic loss of an entire iso-IX. One of these (figure 3) was a triplex-simplex individual mentioned in another paper (SEARS, 1952), and the other was duplex-nulliplex. The latter, which arose from iso-mono-IX

selfed, had an iso-IX in the duplex portion (rather than two telos, for nullisomic offspring were segregated), and was apparently nulli-IX in the nulliplex sector. The former was presumably the same except for the presence of a normal chromosome in both sectors. Either of these two plants may have arisen following misdivision of a telocentric to give a deficient sector and a sector with an isochromosome, but this would have involved the unlikely events of disappearance of the parental type of tissue and persistence of both daughter types in the meristematic region. LINDHARD (1922) reported a „compactum" (triplex) plant one spike of which was half compactum and half heterozygous speltoid (simplex). ISHIKAWA (1934) noted a similar chimera. Four instances of loss of other isochromosomes have been observed in wheat, namely iso-V (figure 4), iso-VII, iso-VIII, and iso-XVIII.

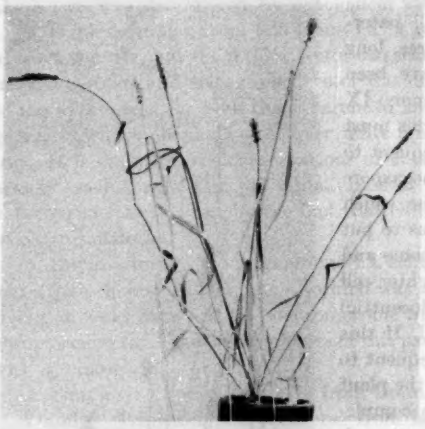

Fig. 3. Chimera in *Triticum aestivum* var. Chinese Spring. Center two spikes have an isochromosome and a telocentric for the long arm of chromosome IX; the other five spikes lack the iso. 0.09 ×.

Previous indications of the somatic loss of one arm of an isochromosome (SEARS, 1946) have not been confirmed. It seems very likely, however, that such losses do occur, because the reciprocal change, addition of a telocentric, has been observed several times. A somatic misdivision of an isochromosome which distributes an iso and a telo to one daughter cell should send only a telo to the other daughter cell.

Somatic loss of part of a telocentric chromosome, such as RHOADES (1940) observed in maize, has not been found for telo-IX, nor have aberrations involving less than a complete arm been noted for iso-IX.

The frequency of somatic aberrations was obtained for two populations in which the plants were vigorous and highly tillered. In one population grown in 1943, 26 plants had a telo-IX, either alone or in company with an iso-IX, and three of these showed a somatic loss of the telocentric. Of 81 isochromosomes, only one was lost somatically, thus tending to confirm RHOADES' (1940) conclusion that isochromosomes are less prone to undergo somatic aberration than are telo-

centrics. In another population, grown in 1945, two telocentrics out of 15 and one isochromosome out of 10 suffered somatic loss. In this population, there were three plants in which an isochromosome presumably gave rise to an added telocentric during the development of either the gametophyte or the early sporophyte.

It is probable that many of the chimeras observed in wheat by other investigators were due to somatic loss of telocentric IX. ÅKERMAN (1920, 1927) and KAJANUS (1923) noted plants part duplex and part simplex for the speltoid complex of characters, while NILSSON-LEISSNER (1925) observed a simplex-nulliplex chimera for speltoidy. These chimeral plants arose either in mono-IX lines or in intervarietal or interspecific hybrid material, where the presence of telocentric chromosomes, resulting from misdivision of univalents, was to be expected.

Fig. 4. Chimera in *Triticum aestivum* var. Chinese Spring. Most of the plant is nullisomic for chromosome V, but the tall, fertile spike toward the left has an isochromosome for the long arm of V. 0.13 ×.

ÅKERMAN and MAC KEY (1948) reported that one of their speltoid lines gave rise to 0.16% chimeral plants, each plant being part normal (duplex) and part heterozygous speltoid (simplex). These chimeras were assumed to be the result of non-disjunction of a normal monosome IX, but loss of a telocentric from plants with both a normal and a telo-IX seems much more probable. Although the frequency of chimeras in this line was very high in comparison with the other Swedish material, it may have been no higher than in comparable material of the variety Chinese Spring. There is no way of estimating the number of plants with telocentrics in the Swedish material, except by using the relative frequency of isochromosomes and telocentrics produced in Chinese, which is about 3 telocentrics to 2 isochromosomes (SEARS, 1952, table 5). Since there were 63 subcompactoids, each presumably with an isochromosome, the expected frequency of telocentrics would have been not quite 100. But there is evidence that with a lower rate of misdivision such as occurs in this material, fewer isochromosomes are produced relative to telocentrics (SEARS, 1952). The cytological data of SÁNCHEZ-MONGE and MAC KEY (1948) on the Swedish material confirm the relatively low frequency of

isochromosomes to be expected. Thus the actual number of plants with telocentrics may have been several hundred, and the 47 observed with chimeras may not have been an inordinate number. Of 41 plants of Chinese with a telocentric, 5 showed somatic loss of the telocentric.

Discussion.

It is now fairly clear that isochromosomes and telocentrics in wheat behave like normal chromosomes in most respects. When a homologue is present, the telocentric pairs with it at meiosis with only slightly reduced frequency if the arm concerned is of substantial length, and passes normally through the succeeding stages of meiosis. If the telocentric has no homologue, it behaves very much like a normal univalent, undergoing misdivision in some cells to form isochromosomes. The rate of misdivision is possibly somewhat higher for the telocentric than for the corresponding normal chromosome, although no statistically significant difference has yet been shown.

Isochromosomes also pair with a normal homologue (or another isochromosome) if one is present, but they pair in only 50% or less of the sporocytes, since the two arms of the isochromosome tend to pair with each other. Following pairing with a normal chromosome, the isochromosome goes through meiosis in normal fashion. When an isochromosome is univalent, it ordinarily pairs with itself, but otherwise behaves like a normal univalent; possibly its rate of misdivision is somewhat reduced, but this is by no means certain. Both telocentrics and isochromosomes result from misdivision, as with normal chromosomes, but here only the telocentrics are recognizable.

Telocentrics and isochromosomes in wheat go through mitoses with only rare aberrations. However, isochromosomes may occasionally be lost, may give rise to an added telocentric, and probably may lose one arm. Telocentrics may be lost or may give rise to isochromosomes. The data at hand suggest that telocentrcis are more liable to loss than are isochromosomes.

It is probable that if somatic loss of part of a telocentric chromosome occurs in wheat, it does so at a lower frequency relative to complete loss than was true for RHOADES' (1940) telocentric in maize. Of the five chimeras analyzed by RHOADES, only one had lost the entire telocentric, whereas all five of the duplex-simplex chimeras observed in wheat had apparently lost the entire telocentric, for they had lost both of two genes 65 crossover units spart. One instance has been observed of aberration of telo-IX involving other than loss or duplication of the entire telocentric, but this aberration may have occurred at meiosis rather than at mitosis. Similarly, the transmissible dicentric chromosome reported by SEARS and CÂMARA (1952) arose from iso-VII through some process other than simple loss or duplication, but an origin at meiosis seems most probable.

RHOADES' (1940) experiments yielded no evidence that isochromosomes might be formed from telocentrics in somatic tissue of maize. At the first microspore division, however, isochromosomes presumably were produced from telocentrics, for they were carried by an appreciable fraction of the functioning pollen grains in spite of the expected severe selection against pollen with an isochromosome. Through misdivision of the telocentric at the first microspore mitosis, followed by passage of the newly produced isochromosone to the generative nucleus, pollen grains were presumably produced which transmitted the isochromosome but which had a normal vegetative nucleus and hence competed successfully with fully normal pollen. Actually, twice as many isochromosomes were transmitted through the pollen as through the egg, and this suggests that misdivision occurred more frequently at the microspore division than at the comparable stage of egg production. The rate must be low at other somatic stages, for no isochromosome-carrying sectors large enough for RHOADES to detect occurred in nearly 200 plants with a telocentric. The single instance observed in wheat of post-meiotic conversion of a telocentric to an isochromosome may have occurred in the female gametophyte, or it may have taken place in sporophytic tissue. In the latter case it must have occurred very early in development, for every spike produced by the plant apparently carried the isochromosome.

Summary.

Genetic tests with chromosome IX of common wheat showed 13.3% formation of isochromosomes from telocentrics as against 7.0% for the same long arm of normal IX, and 7.0% formation of telocentrics from each arm of iso-IX instead of 10.8% from the same arm of normal IX. The differences are not significant statistically.

No differences in frequency of formation of isochromosomes were found among 16 independently produced telocentrics for the long arm of chromosome IX.

Somatic loss of both telocentrics and isochromosomes for chromosome IX and other wheat chromosomes has been observed, with loss of the telo being somewhat more frequent. A telocentric gave rise to an isochromosome somatically, and isochromosomes added a telocentric. Two complex aberrations involving loss and duplication of parts of arms were observed, one of a telo and one of an iso; but these aberrations probably occurred at meiosis.

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DESOXYRIBOSE NUCLEIC ACID IN EMBRYONIC DIPLOID AND HAPLOID TISSUES.

By

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With 2 figures in the text.

(Eingegangen am 15. Oktober 1951.)

Introduction.

Constancy in the amount of desoxyribose nucleic acid in the cells of various tissues of an individual was first reported by BOIVIN, VENDRELY and VENDRELY (1948), who further suggested that such a constant relationship might be an expression of the genic equipment of the cells. Supporting evidence has more recently been brought forward in other investigations. Here may be listed the work of VENDRELY and VENDRELY (1949, 1950), SWIFT (1950a, 1950b) and ALFERT (1950) and in part the findings of MIRSKY and RIS (1949). The apparent exceptions reported by the last named authors for certain beef tissues has been adequately explained as due to polyploidy (LEUCHTENBERGER, VENDRELY and VENDRELY 1951). In general, therefore, the concept of BOIVIN, VENDRELY and VENDRELY rests on a good foundation, at least in so far as adult tissues are concerned. However, some exceptions to this constancy have been reported in the plant *Tradescantia* (SCHRADER and LEUCHTENBERGER 1949), in the rat (PASTEELS and LISON 1950) and in developing sea urchins (LISON and PASTEELS 1951). The hypothesis of LISON and PASTEELS that some variation in DNA is associated with morphogenetic processes is of special interest, and the exceptional cases may thus throw light on the relation of DNA to differentiation as well as to the transmission of hereditary characters.

It was hoped that an analysis of the BOIVIN, VENDRELY and VENDRELY concept would be furthered by an investigation of individuals in which the fundamental nuclear conditions had been altered experimentally. One means of bringing this about lies in the production of haploidy in an animal that normally is diploid. The present paper is concerned with the cytochemical examination of such haploid and normal diploid embryos in frogs. This material offered the additional advantage of permitting an analysis of the hypothesis of LISON and PASTEELS, since a study was made of the range of DNA that is associated with embryonic development.

I wish to express my gratitude to Professor A. W. POLLISTER, in whose laboratory this work was done. In addition I wish to thank Dr. C. LEUCHTENBERGER for her collaboration in the initial phases of this work and her assistance and advice throughout its course.

Materials and Methods.

Eggs were obtained by pituitary induced ovulation from one *Rana pipiens* SCHREIBER female and fertilized by the combined sperm of two males of the same species. Using the method of PORTER (1939), the maternal chromosomes were removed with a glass needle. A small exovate containing the second maturation division spindle and chromosomes is formed. This exovate ultimately becomes completely detached from the egg surface and the operated egg develops into a haploid embryo, with only the paternal chromosomes. This method is a simple and effective way of obtaining haploid embryos (PORTER 1939, MOORE 1950).

Operated and unoperated eggs were placed in separate bowls and kept at 20° C. until hatching. By this time the haploid embryos were markedly retarded and were easily recognizable by their characteristic development (PORTER 1939). As it was desired to study diploid and haploid embryos in approximately the same stage of differentiation, a small group of diploid embryos were retarded in their development by placing them at 17° C. and then at 15° C. On the 7th day these diploid embryos had reached stage 20 (gill circulation, SHUMWAY 1940) and since the haploid embryos appeared to be in approximately the same stage of differentiation, individuals from the experimental and control groups were fixed. On the 8th day all diploid embryos were placed at 20° C. On the 9th day diploid embryos which had been at 20° C. constantly, were in stage 24, when the operculum has overgrown the gills on one side. Some of these diploid embryos and those haploid embryos which appeared to be in a similar stage of development to the diploids were fixed. On the 11th day only 12 haploid embryos were surviving out of the original 110, and all were fixed together with 12 diploid embryos which were in stage 25. The latter stage is characterized by an operculum which has overgrown the gills on both sides.

The haploid embryos used in the following study are believed to be haploid in all their tissues. Their external appearance was characteristic of haploid *R. pipiens* embryos. Their internal differentiation as studied in the sectioned material was characteristically haploid. Chromosomal counts of metaphase plates showed each embryo to be haploid. PORTER (1939) has noted that the haploid nuclear condition remains unchanged until the final stages of development of androgenetic *pipiens* embryos, and then it is altered by the presence of a very few diploid nuclei.

The haploid and diploid control embryos were fixed simultaneously in Carnoy's acetic alcohol (1:3) for 3 hours, washed in absolute alcohol, cleared in benzene and

imbedded in paraffin. The embryos were serially sectioned at 12 μ. A haploid embryo and its diploid control were mounted together on the same series of slides. Staining was by the Feulgen method according to STOWELL (1945). After 12 minutes of hydrolysis all slides were stained for 1 hour with the same preparation of basic fuchsin. An unhydrolyzed control slide was run in each Coplin jar. Thus uniformity of treatment during staining was maintained for all embryos. Any differences, therefore, found in DNA values cannot be ascribed to variations in staining technique. Not only was uniformity of staining of different slides maintained but DNA values of many embryonic tissues were determined from the same slide. For example, all diploid and haploid cartilage and forebrain measurements of the 11 day embryos E were made from the same slide.

The photometric measurements of whole interphase nuclei were made by the method originally described by POLLISTER and RIS (1947). A description of the apparatus in its present form and a discussion of the photometric technique has been given by LEUCHTENBERGER (1950) and SWIFT (1950a).

The DNA per nucleus was estimated after the Feulgen reaction from measurements of the absorption of the 546 mμ line isolated from a G.E. AH4 Mercury vapor lamp by either a Wratten no. 62 filter or a Farrand no. 2756 filter with a peak wave length of 549 mμ and a half band width of 12 mμ. The Wratten filter was used in the preliminary measurements, and the Farrand filter in all later measurements, and the Farrand filter in all later measurements. In each case the transmission through a central cylinder (plug) of the whole resting nucleus was measured and the DNA per nucleus expressed in arbitrary units on the basis of computations as described by SWIFT (1950):

$$\text{amount of DNA in arbitrary units} = \frac{E\, r^2 \text{ plug}}{\text{percent volume}}$$

where E equals the extinction of the plug, r^2 equals the squared radius of the measured area, and the percent volume equals the fraction of the total volume measured. Major and minor axes of the nuclei were measured and the mean considered as the diameter.

In the preliminary measurements a constant plug size of 5 19 μ through the center of each nucleus was measured and the DNA values computed. All later measurements were made with a plug size bearing a constant ratio to the nuclear volume. When using a constant plug size, the percentage of the nuclear volume measured is obviously less in the case of a large nucleus than in a small one. If an essentially constant fraction of the volume of each nucleus is measured, errors that may arise due to differences in chromatin distribution in different nuclei may be avoided. A plug size 60% of the average nuclear diameter was used in all later measurements, following the suggestion of ALFERT (1950). Such a plug size in the tissues here considered is equivalent to 49% of the volume of a nucleus.

Results of the photometric measurements.

Preliminary DNA measurements were made on 2 seven day diploid embryos, 2 seven day haploid embryos, 1 nine day diploid embryo, 1 nine day haploid embryo, 1 eleven day diploid embryo and 1 eleven day haploid embryo. In the 7 day embryos the DNA values of three tissues, representing the three germ layers, were determined. These tissues were forebrain, pronephros and liver. In the 9 day embryos nuclei from these same three tissues, with the addition of cartilage, were measured. In the eleven day embryos nuclei of the same tissues were

measured as in the 9 day embryos, and in addition, those of red blood cells and heart.

The results are presented in Table 1, where the diploid and haploid embryos which were mounted together on the same series of slides appear in parallel columns. It is evident that the range of DNA values is large in both diploid and haploid tissues. The average amount of DNA is higher in the diploid tissues than in the haploid tissues but has no constant relationship, as the ratios of diploid to haploid amounts of DNA varies from 1·34:1 to 2·26:1.

From the above data it seemed that the range of DNA values might be less in some of the older tissues than in the younger tissues. Therefore large numbers of nuclei of the different tissues of the 7 day diploid embryo A and the 7 day haploid embryo A were measured and compared with measurements obtained from an 11 day diploid embryo E and an 11 day haploid embryo E. The individual measurements were used to construct the histograms of Figs. 1 and 2 and a summary of the data is given in Table 2.

The range of DNA values in Table 2 of both diploid and haploid tissues is similar to the range shown by preliminary measurements (Table 1). In most cases the DNA ratio of diploid to haploid is approximately 2:1, although in the pronephros of the 11 day embryos E it is 1·54:1.

The differences in DNA values between younger and older tissues, suggested by the preliminary measurements, were found to exist. The range of DNA values of the forebrain of the 7 day haploid embryo A and the 7 day diploid embryo A is fairly large (Fig. 1). In the 11 day haploid embryo E and the 11 day diploid embryo E the forebrain values have a narrow range (Fig. 1).

The range of DNA values of the pronephros of the 7 day haploid embryo A and the 7 day diploid embryo A is large (Fig. 1). In the 11 day haploid embryo E the range of DNA values of the pronephros is similar to that of the pronephros of the 7 day haploid embryo A (Fig. 1). In the 11 day diploid embryo E the range of DNA values of the pronephros is greatly reduced when compared with that of the pronephros of the 7 day diploid embryo A (Fig. 1).

The range of DNA values is large in the liver of the 7 day haploid embryo A and the 7 day diploid embryo A (Fig. 2). The range of DNA values in the liver of the 11 day haploid embryo E is similar to that of the liver of the 7 day haploid embryo A (Fig. 2). The range of DNA values in the liver of the 11 day diploid embryo E is essentially the same as that of the liver of the 7 day diploid embryo A (Fig. 2).

It appears from the above description that the amount of DNA per nucleus in the 7 day haploid and diploid embryos is more constant in the

Table 1. *Comparison of desoxyribose nuclei acid in the nuclei of various tissues of diploid and haploid Rana pipiens embryos.*

Table 1. *Comparison of desoxyribose nuclei acid in the nuclei of various tissues of diploid and haploid Rana pipiens embryos.*

Embryo	Tissue	Diploid			Haploid			Ratio of diploid to haploid amounts of DNA
		Number of nuclei measured	Range of DNA values	Average amount of DNA per nucleus	Number of nuclei measured	Range of DNA values	Average amount of DNA per nucleus	
A 7 days old diploid stage 20	Forebrain	10	4·90– 7·57	6·49 ± 0·29	10	2·72–6·02	4·09 ± 0·35	1·59:1
	Pronephros	11	2·94– 8·75	6·33 ± 0·60	10	2·66–7·00	4·79 ± 0·46	1·34:1
	Liver	9	6·21–10·20	7·68 ± 0·53	10	3·02–5·03	3·69 ± 0·23	2·08:1
B 7 days old diploid stage 20	Forebrain	10	4·23– 7·57	5·51 ± 0·10	10	2·89–4·00	3·29 ± 0·11	1·68:1
	Pronephros	10	4·16– 9·13	5·91 ± 0·42	10	2·74–5·23	3·56 ± 0·24	1·66:1
	Liver	11	5·05–10·80	8·01 ± 0·49	10	2·21–4·74	3·55 ± 0·24	2·26:1
C 9 days old diploid stage 24	Forebrain	12	5·10– 7·41	6·24 ± 0·23	12	2·75–6·56	3·71 ± 0·32	1·68:1
	Pronephros	12	5·00– 8·72	6·42 ± 0·30	12	3·31–6·47	4·75 ± 0·23	1·35:1
	Liver	12	4·65–10·70	8·26 ± 0·53	12	3·42–6·16	4·90 ± 0·21	1·68:1
	Cartilage	10	4·13–6·75	5·41 ± 0·24	10	2·84–5·41	3·93 ± 0·25	1·38:1
D 11 days old diploid stage 25	Forebrain	9	5·27– 6·80	5·95 ± 0·17	10	2·61–3·57	3·03 ± 0·10	1·96:1
	Pronephros	10	4·38– 7·11	5·44 ± 0·25	10	2·40–5·87	4·02 ± 0·12	1·35:1
	Liver	11	4·53– 9·93	6·52 ± 0·54	16	2·48–5·53	3·89 ± 0·03	1·68:1
	Cartilage	10	3·36– 5·26	4·45 ± 0·19	10	2·08–4·13	2·80 ± 0·19	1·59:1
	Red blood cells	12	4·05– 9·63	6·04 ± 0·48	12	2·89–4·22	3·49 ± 0·12	1·73:1
	Heart	20	3·39– 8·28	6·03 ± 0·34	22	2·40–6·39	3·59 ± 0·23	1·68:1

Table 2. *A comparison of nuclear DNA in tissues of diploid and haploid 7 day embryos A with diploid and haploid 11 day embryos E.*

Embryo	Tissue	Diploid			Haploid			Ratio of diploid to haploid amounts of DNA
		Number of nuclei measured	Range of DNA values	Average amount of DNA per nucleus	Number of nuclei measured	Range of DNA values	Average amount of DNA per nucleus	
7 day A	Forebrain	38	5·12–10·40	6·79 ± 0·21	39	2·42–5·25	3·35 ± 0·10	2·02:1
11 day E	Forebrain	30	3·72– 7·11	6·11 ± 0·14	30	2·74–4·11	3·40 ± 0·05	1·83:1
7 day A	Pronephros	39	2·68–12·70	6·33 ± 0·29	37	2·22–5·82	3·45 ± 0·14	1·83:1
11 day E	Pronephros	33	4·13– 8·96	5·64 ± 0·15	32	2·15–6·62	3·65 ± 0·21	1·54:1
7 day A	Liver	39	4·81–12·30	7·64 ± 0·32	35	2·36–6·24	3·71 ± 0·21	2·06:1
11 day E	Liver	32	3·96– 9·96	6·75 ± 0·32	34	2·36–6·11	3·38 ± 0·18	2·00:1
11 day E	Cartilage	24	4·95–10·10	6·24 ± 0·28	31	2·53–6·27	3·32 ± 0·14	1·88:1

forebrain tissue than in the pronephros or liver tissues. It is also evident in both haploid and diploid embryos that the amount of DNA is more constant in the 11 day forebrain than in the 7 day forebrain. Similarly, the amount of DNA is more constant in the pronephros of the 11 day diploid embryo than in the pronephros of the 7 day diploid embryo.

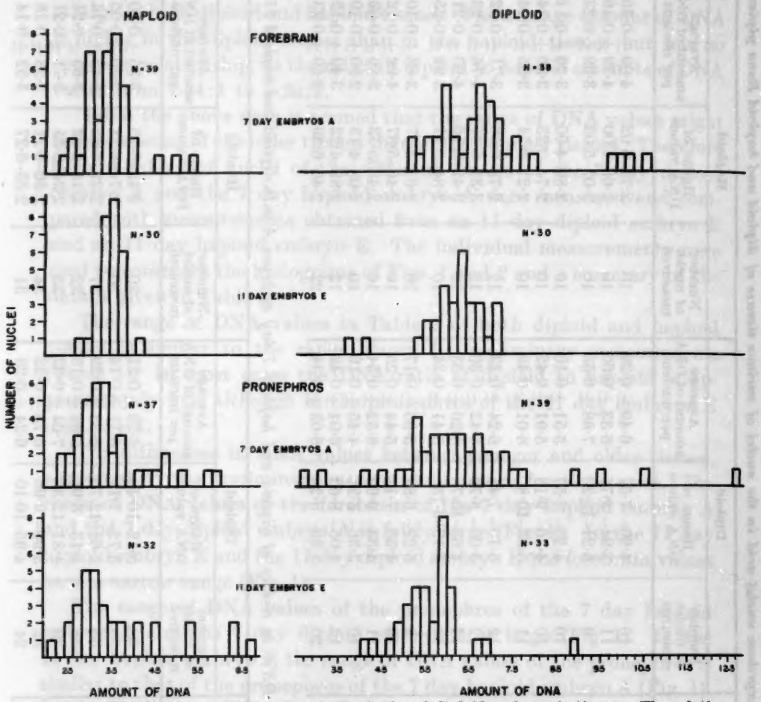

Fig. 1. Distribution of DNA values in haploid and diploid embryonic tissues. The relative amounts of DNA with class intervals of .20 are shown on the abscissa and the number of nuclei measured are shown on the ordinate.

DNA measurements of cartilage (which is absent in the 7 day embryos) are shown in Fig. 2 for the 11 day haploid and diploid embryos E. The range of DNA values is larger than in the forebrain tissue of the same embryos and is somewhat similar to the range in the liver and pronephros.

DNA values were determined for the heart in the 11 day haploid and diploid embryos D (Table 1). In all other haploid embryos heart tissue was very poorly differentiated and was not measured. The range of

DNA values of the haploid heart is similar to that of the liver of the 11 day haploid embryo E (Fig. 2). The range of DNA values of the diploid heart is similar to that of the liver of the 11 day diploid embryo E (Fig. 2).

From the data presented it is apparent that a wide range of DNA values exists in both haploid and diploid embryos. The mean DNA

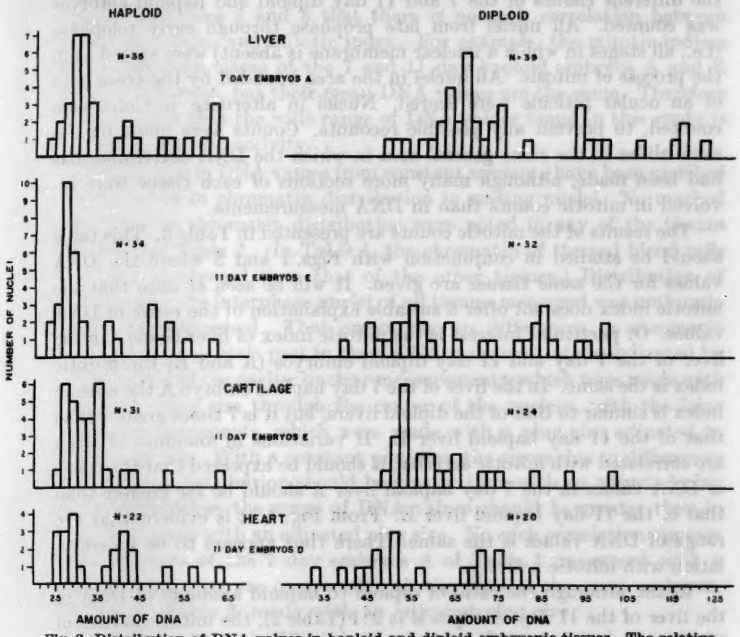

Fig. 2. Distribution of DNA values in haploid and diploid embryonic tissues. The relative amounts of DNA with class intervals of .20 are shown on the abscissa and the number of nuclei measured are shown on the ordinate.

values are markedly higher in the diploid embryos than in the haploid embryos (Table 2). In certain tissues the ratio of diploid to haploid amounts is 2:1 (Table 2), which is the ratio expected from the concept that DNA is associated with the chromosomes. SWIFT (1950) has shown that the distribution of DNA values in embryonic *Ambystoma* and mouse tissues gives a broad curve, similar to some of my histograms. He believes that the large number of intermediate DNA values found in the interphase nuclei of dividing tissues, such as embryonic tissues, is due to the building up of DNA in the interphase nucleus before the

visible onset of prophase. If this interpretation is correct, the mitotic index should be low in the embryonic tissues which have a narrow range of DNA values. the tissues which have a wide range of DNA, and high in values. Moreover, the mitotic index should be the same in diploid and haploid tissues whose ratio of diploid to haploid amounts of DNA is 2:1.

In order to test this interpretation, the number of mitoses present in the different tissues of the 7 and 11 day diploid and haploid embryos was counted. All nuclei from late prophase through early telophase (i.e., all stages in which a nuclear membrane is absent) were scored as in the process of mitosis. All nuclei in the area bounded by the cross lines of an ocular reticule were scored. Nuclei in alternate sections were counted, to prevent any possible recounts. Counts were made on the same slides in the same general area in which the DNA determinations had been made, although many more sections of each tissue were involved in mitotic counts than in DNA measurements.

The results of the mitotic counts are presented in Table 3. This table should be studied in conjunction with Figs. 1 and 2 where the DNA values for the same tissues are given. It will be seen at once that the mitotic index does not offer a suitable explanation of the range of DNA values. Of particular interest is the mitotic index of liver tissue. In the liver of the 7 day and 11 day diploid embryos (A and E) the mitotic index is the same. In the liver of the 7 day haploid embryo A the mitotic index is similar to that of the diploid livers, but it is 7 times greater than that of the 11 day haploid liver E. If variations in amounts of DNA are correlated with mitotic activity, it should be expected that the range of DNA values in the 7 day haploid liver A should be far greater than that of the 11 day haploid liver E. From Fig. 2, it is evident that the range of DNA values is the same. There thus appears to be no correlation with mitotic activity.

Again, although the ratio of diploid to haploid amounts of DNA in the liver of the 11 day embryos E is 2:1 (Table 2), the mitotic indices of the diploid and haploid livers are not the same. This is in direct contra-

Table 3. *The mitotic index of diploid and haploid embryonic tissues.*

Embryo	Tissue	Diploid			Haploid		
		Number of nuclei counted	Number of nuclei in mitosis	Mitotic index %	Number of nuclei counted	Number of nuclei in mitosis	Mitotic index %
7 day A	Forebrain	1271	50	3·93	1682	42	2·50
11 day E	Forebrain	1519	12	0·79	1497	8	0·53
7 day A	Pronephros	702	19	2·71	803	25	3·11
11 day E	Pronephros	922	18	1·95	1023	25	2·44
7 day A	Liver	889	44	4·95	1405	72	5·12
11 day E	Liver	1835	88	4·80	1016	7	0·69
11 day E	Cartilage	2462	29	1·18	1369	4	0·29

diction to expectation, based on SWIFT's hypothesis, and gives additional evidence that variable amounts of DNA are not due to mitotic activity.

Furthermore, if SWIFT's hypothesis is correct, the mean DNA value of rapidly dividing tissue should be higher than that of slowly dividing tissue, since a greater number of intermediate DNA values would be present in the former tissue than in the latter. It is evident from a comparison of Tables 2 and 3 that there is no such correlation between mean DNA value and mitotic index. For example, the mitotic indices of the forebrain tissue of the 7 and 11 day haploid embryos A and E are very different, but their mean DNA values are the same. Therefore it is apparent that the wide range of DNA values found in this study is not due to mitotic activity.

Differences in DNA values from constant amounts have been ascribed to differences in chromatin distribution in resting nuclei. No marked differences in chromatin distribution were noted in any of the tissues measured in Table 2. (In Table 1, the chromatin of the red blood cells was more condensed than that of the other tissues.) Distribution of chromatin in the interphase nuclei of all tissues measured was uniformly and evenly dispersed. That errors due to differences in chromatin distribution play little part in these DNA measurements is indicated by a comparison of the earlier nuclear measurements, which were made with a constant plug size through the center of the nucleus, with the later nuclear measurements, which were made with a plug size adjusted to the nuclear size. With a constant plug size, the errors due to differences in chromatin distribution should be greater than with an adjusted plug size, and therefore the range of DNA values should be greater than in nuclei measured with an adjusted plug size. No such correlation appears when the data of the 7 day embryos A of Table 1, measured with a constant plug size, are compared with the data of the same embryos, recorded in Table 2, made with an adjusted plug size.

LEUCHTENBERGER's (1950) demonstration that a marked decrease in DNA accompanies pycnosis necessitates the consideration of the possibility that different degrees of pycnosis among the different tissues of the haploid and diploid embryos might affect the results of the DNA measurements, although only normal appearing nuclei were measured. In the 7 day embryos A, an occassional pycnotic nucleus was observed in both the diploid and haploid liver, and in the diploid forebrain. More pycnotic nuclei were noted in the haploid forebrain. None were found in the pronephros of the 7 day embryos A. In the 11 day embryos E, no pycnosis was noted in the diploid embryo, but it occurred in all the haploid tissues measured, being particularly great in the liver. Pycnosis has not affected the DNA values of the normal nuclei measured, since they show no decrease in DNA values (Figs. 1 and 2).

Determinations of DNA have been made on adult *Rana pipiens* tissues by SWIFT (1950), using the same photometric method as employed here. Since the DNA values presented here have been calculated according to SWIFT's formula, a comparison of our data is possible. Although different fixatives and different preparations of Feulgen reagent were used, the similarity of the results is noteworthy. SWIFT's DNA value for 20 erythrocyte nuclei is $6{\cdot}00 \pm {\cdot}07$. My DNA value for 12 red blood cell nuclei of the 11 day diploid embryo D (Table 1) is $6{\cdot}04 \pm {\cdot}48$. SWIFT's value for 20 kidney tubule nuclei (mesonephros) is $5{\cdot}60 \pm {\cdot}06$. My value for 33 pronephros nuclei of the 11 day diploid embryo E (Table 2) is $5{\cdot}64 \pm {\cdot}15$. This marked agreement in DNA values is not present in our liver measurements. SWIFT separates his liver values into two classes, Class I with a mean value of $5{\cdot}60 \pm {\cdot}09$ and Class II with a mean value of 10·9. I find the value of $6{\cdot}75 \pm {\cdot}32$ in liver nuclei of the 11 day diploid embryo E (Table 2), and from the histogram of this tissue (Fig. 2) it is obvious that the measurements can not be grouped into 2 classes of DNA values.

In addition to the above measurements on diploid nuclei, SWIFT determined the DNA value in spermatid nuclei, which are haploid and can be compared with the nuclei of my haploid embryos. In 20 *R. pipiens* spermatids SWIFT found the DNA value to be $2{\cdot}80 \pm {\cdot}06$. This corresponds to the lowest mean haploid value that I ever obtained. It is recorded in Table 1, where the cartilage nuclei in the 11 day haploid embryo D had a mean DNA value of $2{\cdot}80 \pm {\cdot}19$. This value is 16% lower than the value of $3{\cdot}32 \pm {\cdot}18$ found in the cartilage of the 11 day haploid embryo E (Table 2), which is my lowest DNA value based on a large number of nuclei.

The mean DNA value of the 235 diploid nuclei recorded in Table 2 is 6.54. The mean DNA value of the 238 haploid nuclei recorded in Table 2 is 3·47. This is a ratio of diploid to haploid amounts of DNA of 1·88:1. The mean volume of these same diploid nuclei is 289 μ^3 and of these same haploid nuclei is 137 μ^3, which is a ratio of 2·11:1. Since the diploid to haploid volume ratio is more than 2:1, and the DNA ratio is less than 2:1, it may be that the larger volume of the diploid nuclei is due to a greater synthesis of proteins in the diploid nuclei than in the haploid nuclei. SCHRADER and LEUCHTENBERGER (1950) have shown such a correlation between increased nuclear volumes and increased total proteins in *Arvelius*. It is interesting to speculate on the role of sucha possible decrease in the synthesis of nuclear proteins in the differentiation of haploid embryos, which develop abnormally and ultimately die.

Although the mean volumes of diploid and haploid nuclei have a ratio of 2·11:1, there is great variation in nuclear sizes. The diploid nuclei recorded in Table 2 ranged from 90 μ^3 to 775 μ^3 in volume. The

haploid nuclei of the same table ranged from 60 μ^3 to 360 μ^3 in volume. Nevertheless, nuclei of different volumes may have the same amount of DNA. For example, in the forebrain of the 11 day diploid embryo E, a DNA value of 5·90 was found in 2 nuclei whose volumes were 300 μ^3 and 607 μ^3. Furthermore, nuclei of the same volume may have different amounts of DNA. Of the diploid nuclei recorded in Table 2, 18 were 300 μ^3 in volume. Their DNA values ranged from 2·68 to 9·97. (Sixty-six percent of these nuclei had DNA values between 5.01 and 6.06.) LEUCHTENBERGER and SCHRADER (1951) have noted the independence of nuclear size and of DNA content in the rat liver but found a direct correlation in the increase in nuclear volume and the amount of nuclear proteins. (They did not find this correlation in the nuclei of cells with different synthesizing abilities.) The relationships of DNA, protein synthesis and nuclear volumes would be of great interest in a study of embryonic tissues.

Discussion.

The data herein presented support, in a general way, the original concept of BOIVIN, VENDRELY and VENDRELY (1948), since the mean amounts of DNA in the different tissues of the same embryo are approximately constant. Moreover, the difference between the mean amounts of DNA in the diploid and haploid embryos, which is approximately 2:1, is to be expected on the concept of DNA being associated with the chromosomes, since embryos with twice the number of chromosomes have approximately twice the amount of DNA per nucleus.

However, the major interest of these data lies in their demonstration of the wide range in nuclear DNA values in both haploid and diploid embryos, and the correlation of changes in this range with progressive differentiation.

It has also been noted by LEUCHTENBERGER, VENDRELY and VENDRELY (1951) that, though the mean DNA amount was constant and the same for diploid nuclei of different tissues of the same animal, there was a variation in the amount of DNA from nucleus to nucleus in each tissue, sometimes as high as 50%.

It is becoming increasingly clear that such differences in DNA values are in a large part due to real biological variation and not, as was earlier assumed, due to errors inherent in the cytochemical photometric method.

Amounts of DNA twice and four times that of the diploid amount have been described by RIS and MIRSKY (1949), PASTEELS and LISONS (1950), SWIFT (1950) and LEUCHTENBERGER, VENDRELY and VENDRELY (1951). Such multiple differences in DNA values have been shown to be associated with polyploidy.

The wide range of DNA values of the data presented can not be explained on the basis of a polyploid series. An examination of the

histograms shows that in the haploid tissues there is no grouping of nuclei into those with diploid amounts of DNA, and in the diploid tissues there is no grouping of nuclei into those with tetraploid amounts of DNA. Moreover, not one case of polyploidy nor any visible expression of abnormal polyteny was encountered in the 260 diploid and 183 haploid mitoses observed during the determination of the mitotic index for the various tissues.

Variations in DNA values in embryonic tissues have been described by SWIFT (1950) and attributed to the building up of DNA in tissues dividing at different mitotic rates. From the data presented herein the broad range of DNA values is not correlated with the mitotic index.

LISON and PASTEELS (1951) interpret the marked fluctuations in nuclear DNA which they find in developing sea urchin eggs to morphogenetic activity, and not to the influence of mitosis. The data I have here presented further indicate a correlation between a wide range of DNA values and the differentiation of embryonic tissues. For example, forebrain is one of the earliest tissues to differentiate and there is a marked difference in the degree of differentiation of the young forebrain of a 7 day and of an 11 day *R. pipiens* embryo. From the histograms of forebrain (Fig. 1) it is seen that there is a marked corresponding difference in the range of DNA values in the forebrain of a 7 day and of an 11 day embryo. The nuclei of the well differentiated forebrain tissue of the 11 day embryos show a much narrower range of DNA values than do those of the 7 day embryos. Moreover, in the 7 day embryos the range of DNA values in the forebrain is less than that of the pronephros or the liver (Figs. 1 and 2). This is also true of the 11 day embryos and may be correlated with the fact that the forebrain differentiates earlier than the other tissues studied.

The values of DNA in the pronephros (Fig. 1) show a similar relationship as in the forebrain between the younger and older diploid embryos. The haploid pronephros, however, has the same spread in both younger and older embryos. It may well be that the haploid pronephros has not reached the same stage of differentiation as the diploid pronephros, since haploid embryos are retarded in their development.

The liver DNA values (Fig. 2) show a large range in both younger and older haploid and diploid embryos. The haploid liver of the 7 day embryo was loose and spongy in appearance, whereas the diploid control liver was still thick and yolky, so that actually the diploid liver was at an earlier stage of differentiation than the haploid liver. The 11 day haploid liver has increased in size but appears similar to the 7 day haploid liver, and the range of DNA values is similar. The 11 day diploid liver has become loose and spongy, in contrast to the 7 day diploid liver, and has grown tremendously in size. This differentiation may be reflected in the somewhat smaller range of DNA values in the 11 day diploid liver.

From these data it appears that a wide range of DNA values may be correlated with differentiation. As differentiation proceeds, the range of DNA values may well become less and ultimately, in an adult organism, the DNA values may fluctuate only slightly about a constant value. Such an interpretation appears justified on the basis of a comparison of SWIFT's (1950) DNA values in adult *R. pipiens* with my DNA values in embryonic *R. pipiens*. Not only is SWIFT's spermatid DNA value lower than my mean haploid value, but also his DNA values for various tissues are lower than my mean diploid value. His standard errors are also lower than mine. This is supporting evidence for the possibility that in adult tissues the DNA content settles down to a more constant value than that found in differentiating tissues.

The work of REISNER and KORSON (1951) also lends support to this hypothesis. Although the authors do not emphasize the point, their data clearly show a decrease in the amount of nuclear DNA with the progressive differentiation of the erythroblasts of normal adult man.

It may well be that during differentiation DNA may be produced in variable amounts at different regions of the chromatin. Certain specific regions may be associated with the differentiation of specific tissues. These regions may produce large quantities of DNA, so that the mean N or 2 N value of DNA is not present in all nuclei at any one time, but in variable amounts, sometimes larger, sometimes smaller, depending on whether the regions responsible for the differentiation of a specific tissue are actively producing DNA or not. In older, well differentiated tissues, DNA values become more constant, although still variable, as noted by LEUCHTENBERGER, VENDRELY and VENDRELY (1951) in their photometric measurements of beef tissue. Such variability in adult tissue may be associated with the maintenance of function.

It was suggested by BOIVIN, VENDRELY and VENDRELY (1948) that DNA may be the genic material. If DNA is the genic material or an essential component thereof, yet is variable in amount in differentiating tissues, perhaps we have here an explanation of how cells with the same chromosome complement differentiate into markedly different tissues. The genes controlling certain morphogenetic processes may produce different amounts and kinds of DNA at different times, resulting in differentiation.

Summary.

Nuclear DNA values were determined by the cytochemical photometric method for various tissues of diploid and haploid *Rana pipiens* embryos. It was found that the ratio of diploid to haploid amounts of nuclear DNA was approximately two to one.

The major interest of this study lies in the demonstration of a wide range of nuclear DNA values and the correlation of changes in this range with progressive differentiation. Older, more differentiated tissues

were found to have a narrower range of DNA values than younger, less differentiated tissues.

Evidence is cited that such differences in DNA values are real biological variations and the results are discussed in relation to differentiation.

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DIE ERZEUGUNG VON CHROMOSOMENDISLOKATIONEN DURCH RÖNTGENSTRAHLEN IN DER KEIMBAHN UND IN GESCHLECHTSZELLEN VERSCHIEDENEN ALTERS BEI *DROSOPHILA HYDEI* STURT.

Von

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(Eingegangen am 21. Oktober 1951.)

Die Entstehung einer strahleninduzierten, gröberen Chromosomendislokation resultiert aus zwei verschiedenen Vorgängen: der Entstehung von mindestens zwei Primärbrüchen innerhalb eines Kerns und der dislozierten Verbindung der Bruchstellen. Der Vorgang der Entstehung eines Primärbruches ist als Eintreffereignis experimentell gut gesichert. Inwieweit er vom physiologischen Zustand der Zelle und der Chromosomen abhängt, kann bisher noch nicht entschieden werden. Für den zweiten Vorgang ist eine solche Abhängigkeit mit Sicherheit anzunehmen, da die Lageverhältnisse der Chromosomen im Kern, ihr Spiralisierungsgrad und ähnliches sicher auf die Häufigkeit und die Art dislozierter Verheilungen einen großen Einfluß haben müssen. In den meisten bisherigen Versuchen mit *Drosophila* wurden die Dislokationen durch Bestrahlung reifer Spermien induziert. Die vorliegenden Versuche sollen einen Beitrag zu der Frage liefern, inwieweit Dislokationen durch die Bestrahlung anderer Stadien der Keimbahn erzeugt werden können.

Als Methode wurde der cytologische Nachweis der Dislokationen in den Speicheldrüsenchromosomen der Larven gewählt, die aus der Paarung bestrahlter Tiere mit unbestrahlten hervorgehen. Durch diese Methode werden alle gröberen Dislokationen erfaßt, die durch die Geschlechtszellen der bestrahlten Tiere übertragen werden und die im heterozygoten Zustand den Ablauf der Mitosen und der Entwicklung bis zur erwachsenen Larve nicht behindern, mit Ausnahme der intrachromosomalen Dislokationen des X-Chromosoms in männlichen Larven und der Dislokationen, die sich nur auf rein heterochromatische Chromosomenschenkel beschränken. Die meisten bisherigen Untersuchungen verwenden zum Nachweis der strahleninduzierten Dislokationen genetische Methoden. Diese sind zwar leichter zu handhaben, gestatten aber nur den Nachweis gewisser Dislokationsbereiche in bestimmten Chromosomen.

Als Objekt wurde *Drosophila hydei* STURT. gewählt. Der verwendete Stamm war von einem in Schladming (Steiermark) gefangenen Weibchen

abgeleitet und erwies sich bei wiederholter Prüfung als strukturell völlig homozygot. Diese Art bietet den Vorteil, in der Kultur sehr langlebig und bis über zwei Monate voll fruchtbar zu sein. Außerdem ist sie sehr zuverlässig in der Kultur und besitzt einen sehr übersichtlichen Riesenchromosomensatz aus dicken, gut färbbaren und leicht zu identifizierenden Elementen. *Drosophila hydei* ist in den natürlichen Populationen fast vollkommen frei von Dislokationen. WARTERS (1944) fand bei der Untersuchung von vielen tausend Individuen aus verschiedenen Teilen des über die ganzen gemäßigten Zonen der Erde ausgebreiteten Wohngebietes nur eine einzige Inversion. Unser Stamm erwies sich bei Kreuzung mit einem Stamm aus Nordamerika (Kollektion SPENCER) als strukturell mit diesem völlig identisch. Diese hochgradige strukturelle Stabilität ist bei *Drosophila*-Arten nicht häufig. Es ist nicht zu entscheiden, ob sie auf der großen Seltenheit spontaner Dislokationsentstehung oder auf einer sehr wirksamen negativen Auslese der Dislokationen beruht. Jedenfalls empfiehlt auch dieser Umstand die Art für Strahleninduktionsversuche.

Die cytologischen Verhältnisse von *Drosophila hydei* sind durch die Arbeiten von BAUER (1936) und WHARTON (1943) aufgeklärt. Der Satz der Mitose-Chromosomen zeigt 4 stabförmige und 1 kleines kugelförmiges Autosomenpaar, sowie ein Geschlechtschromosomenpaar, das beim Weibchen aus zwei V-förmigen X-Chromosomen, beim Männchen aus einem V-förmigen X-Chromosom und einem J-förmigen Y-Chromosom besteht. Die Riesenchromosomen zeigen 5 lange Elemente entsprechend den 4 langen Autosomen und dem einen Schenkel des X-Chromosoms und ein kurzes Element entsprechend dem kugelförmigen Chromosom. Der zweite Schenkel des X-Chromosoms und das ganze Y-Chromosom sind heterochromatisch. Sie bauen zusammen mit heterochromatischen Anteilen der Spindelfaseransatzzone der anderen Chromosomen das große Chromozentrum auf. Es ist eine Eigentümlichkeit von *Drosophila hydei*, daß die vier langen autosomalen Elemente stets mit ihren distalen Enden in einem Punkt zusammenhängen, während das etwas kürzere Element des X-Chromosoms an dieser Verbindung meist nicht teilnimmt (BAUER). Bei flüchtiger Betrachtung kann diese Verbindungsstelle eine Dislokation vortäuschen. Das X-Element ist bei männlichen Larven nicht dünner, aber deutlich schwächer gefärbt als bei weiblichen. Es wurde eine Chromosomenkarte von *Drosophila hydei* entworfen, auf deren Wiedergabe hier verzichtet wird. Im Gesamtcharakter ähneln die Riesenchromosomen denen von *Drosophila repleta* (Karte bei WHARTON, 1942). Sie zeigen deutliche Marken, so daß ihre Identifizierung leicht möglich ist. Die Paarungsaffinität ist gut, spontane Paarungslücken sind nicht sehr häufig und betreffen bevorzugt das X-Chromosom, das bei weiblichen Larven in manchen Kernen ganz

ungepaart sein kann. Auch die Dislokationen sind im heterozygoten Zustand meist sehr exakt gepaart und daher leicht zu deuten.

Eine Analyse der einzelnen strahleninduzierten Dislokationen lag nicht in der Absicht dieser Arbeit. Die Bruchstellen erscheinen, soweit sich dies aus unserem Material erschließen läßt, rein zufällig über die Chromosomen verteilt und betreffen gelegentlich auch die heterochromatischen Zonen. Im Protokoll wurde nur zwischen Inversionen und Translokationen unterschieden. Soweit man aus dem zahlenmäßig geringen Material unserer Versuche schließen kann, können wir die Angabe BAUERS (1939) bestätigen, daß die Häufigkeit der reziproken Translokationen sich zu der der Inversionen bei Zweibruchereignissen nach Bestrahlung reifer Spermien etwa wie 2:1 verhält, ein Verhältnis, das gegenüber der Zufallserwartung zugunsten der homosomalen Dislokationen verschoben ist. Eine Defizienz wurde nur einmal beobachtet. Es kamen auch mehrfache Dislokationen vor, z. B. eine Translokation und eine Inversion in einer Larve. Bei der kompliziertesten Figur unseres Materials, einer mehrfachen Translokation, sind mindestens 12 Bruchstellen anzunehmen. In zwei Präparaten wurden Kerne mit je einer Translokation (die eine mit Beteiligung des Heterochromatins) und daneben Kerne ohne Dislokation gesehen. Nach der Lagerung im Präparat ist es wahrscheinlich, daß die eine Speicheldrüse die Dislokation enthielt, die andere nicht. Die Erklärung für diese Erscheinung ist wohl in der Annahme zu suchen, daß die Chromosomen in den Spermien bereits in zwei Chromatiden geteilt sind und daß in diesen Fällen nur die eine Chromatide eine Dislokation erlitten hat. Die Folge wäre eine Larve, die halbseitig die Dislokation zeigt. Strahlengenetische Befunde der letzten Zeit legen die Annahme sehr nahe, daß die Chromosomen im reifen Spermium stets als Doppelchromatiden bestehen.

Bestrahlt wurde mit einem Siemens-Monopan, 4 mA, 60 kV, Tubus 1,5 cm. In der Ebene der flachen Bestrahlungskammern, in der sich die Fliegen aufhalten, war die Leistung 1200 r je 1 min. Die Nachkommenschaft aus der Kopula zwischen bestrahlten und unbestrahlten Tieren wurde stets fraktioniert abgefangen und, wo nicht anders vermerkt, wurde nur die erste Fraktion zur Untersuchung verwendet. Erfaßt wurde damit das Gelege in einem Zeitraum von 2—3 Tagen nach Beginn der Eiablage. War die Zahl der Larven zu gering, wurde das Gelege des 4.—6. Tages hinzugenommen. Die Präparate, in denen mindestens eine Dislokation vorkommt, werden im folgenden als Prozentsatz der Gesamtzahl der verwertbaren Präparate angegeben. Dabei wird keine Rücksicht darauf genommen, wieviel Bruchstellen zum Zustandekommen der betreffenden Dislokationen angenommen werden müssen.

Die Resultate nach der Bestrahlung von Imagines sind in der folgenden Tabelle 1 vereinigt.

Der Unterschied zwischen den Resultaten der Versuche I und II ist nicht statistisch gesichert. In beiden Fällen handelt es sich um die Dislokationsrate, die bei der Bestrahlung von reifen Spermien entsteht.

Tabelle 1.

Versuchsnummer	Versuchsbedingungen	Zahl der geprüften Larven	Davon mit Dislokationen	% ± m
I	1—5 Tage alte Männchen, mit 4800 r bestrahlt, sofort mit unbestrahlten Weibchen gepaart	97	27	27,8 ± 4,6
II	30 Tage alte Männchen, mit 4800 r bestrahlt, sofort mit unbestrahlten Weibchen gepaart	49	11	22,5 ± 6,0
III	Die Männchen aus Versuch I werden nach 30 Tagen von den Weibchen entfernt und mit neuen unbestrahlten Weibchen gepaart (1. und 2. Fraktion)	143	1	0,7 ± 0,8
IV	1—5 Tage alte Männchen, mit 4800 r bestrahlt, werden erst 28 Tage nach der Bestrahlung mit unbestrahlten Weibchen gepaart	98	1	1,0 ± 1,0
V	1—5 Tage alte Männchen, mit 4800 r bestrahlt, werden nach der Bestrahlung für 37 Tage bei einer Temperatur von 8—10° gehalten, dann bei Zuchttemperatur mit unbestrahlten Weibchen gepaart (1. und 2. Fraktion)	72	3	3,2 ± 2,0
VI	1—5 Tage alte Weibchen, mit 4800 r bestrahlt, werden sofort mit unbestrahlten Männchen gepaart (1. und 2. Fraktion)	89	4	4,5 ± 2,3

Diese sind auch schon bei jungen Männchen in großer Menge vorhanden. Die Rate ist mit der von BAUER (1939) bei *Drosophila melanogaster* und von KOLLER und AHMED (1942) bei *D. pseudoobscura* mit der gleichen Methode geprüften Dislokationsrate vergleichbar. BAUER fand für 4000 r eine Rate von 32,31 ± 2,21%, bei 5000 r von 44,9 ± 2,02%. KOLLER und AHMED fanden bei 4500 r eine Ausbeute von 40%. Daß bei *D. melanogaster* und besonders bei *D. pseudoobscura* die Ausbeute an Dislokationen bei gleicher Dosis deutlich höher ist, als in unseren Versuchen mit *D. hydei*, könnte mit der größeren cytologischen Gesamtlänge des Chromosomensatzes bei jenen Arten zusammenhängen.

In Versuch III ist die Ausbeute an Dislokationen äußerst gering. Die Spermien, die diese geringe Dislokationsrate übertrugen, waren zur Zeit der Bestrahlung wahrscheinlich erst im Stadium junger Spermatocyten 2. Ordnung. Daß in diesen Zellen durch die Bestrahlung viel weniger Primärbrüche entstehen als in reifen Spermien, erscheint uns wenig wahrscheinlich. Viel näher liegend ist die Annahme, daß Neukombinationen durch dislozierte Verheilung in ihnen in viel geringerer Häufigkeit zustande kommen als nach Bestrahlung reifer Spermien.

Im Spermienkopf sind die Chromosomen sehr eng und wahrscheinlich parallel gelagert und die Verheilung der Primärbrüche findet, wie aus verschiedenen Versuchsresultaten erschlossen werden konnte, während der Auflockerung des Spermienkerns zum männlichen Vorkern statt, während eines Stadiums also, in dem die Chromosomen aus ihrer sehr engen Parallellagerung sich allmählich umlagern. Die Kerne der Spermatocyten sind groß und locker gebaut und die Chromosomen liegen in ihnen weit voneinander entfernt. Aus diesen Gründen ist anzunehmen, daß in ihnen nur wenig Gelegenheit zur dislozierten Vereinigung von Bruchstellen besteht. CATSCH und RADU (1943a) untersuchten die gleiche Frage bei *D. melanogaster* mit einer genetischen Methode, die die Erfassung der Translokationen zwischen dem 2. und dem 3. Chromosom gestattete. Die Dislokationsrate betrug am 1. Tag nach der Bestrahlung 11,96 $\pm$ 0,64%, um dann allmählich abzusinken, für den 14.—19. Tag auf 1,37 $\pm$ 0,30%, für den 20.—25. Tag auf 0,10 $\pm$ 0,10%. Die Unterschiede liegen in der gleichen Größenordnung wie bei unseren, mit cytologischer Methode durchgeführten Versuchen. CATSCH und RADU erörtern außer der oben gegebenen Deutung für das Absinken der Dislokationsrate auch noch die Möglichkeiten einer Gonenselektion und einer Elimination von Dislokationen bei der Meiose, falls bestrahlte Spermatogonien oder Spermatocyten 1. Ordnung beteiligt sind. Diese Deutungsmöglichkeiten haben wohl wenig Wahrscheinlichkeit für sich. Aus älteren Untersuchungen geht eindeutig hervor, daß für die Ausbeute an strahleninduzierten Dislokationen nach Bestrahlung reifer Spermien der Zeitfaktor keine Rolle spielt, einer der Beweise für die Verlegung der Neukombinationsvorgänge in die Vorkernphase. CATSCH und RADU (1943b) konnten zeigen, daß bei der Bestrahlung von Spermatocyten oder Spermatogonien der Zeitfaktor von Einfluß auf die Rate ist. Auch dies spricht dafür, daß Zustand und Lagerung der Chromosomen im Ruhekern dieser Zellen im Gegensatz zum Spermienkern für den verschiedenen Ablauf der Neukombinationsvorgänge von Bedeutung sind.

Der Versuch IV ergab die gleiche geringe Dislokationsrate wie der Versuch III, obwohl die verwendeten Männchen keine Gelegenheit hatten, die während der Bestrahlung vorhandenen reifen Spermien in der Wartezeit zu verbrauchen. Dies spricht dafür, daß die reifen Spermien in den männlichen Genitalwegen eine beschränkte Lebensdauer haben und ständig durch neugebildete Spermien ersetzt werden, auch dann, wenn kein Verbrauch der fertigen Spermien stattfindet. Auch hier besteht die Denkmöglichkeit eines selektiven Absterbens der mit Brüchen behafteten Spermien als eine sehr unwahrscheinliche Alternative. In Versuch V wurde versucht, durch niedere Temperatur eine Konservierung der bestrahlten reifen Spermien zu erzielen.

Die Dislokationsrate ist zwar etwas höher, doch ist der Unterschied statistisch nicht genügend gesichert. CATSCH, KANELLIS und RADU (1943) ließen die Spermien in den weiblichen Geschlechtswegen von *D. melanogaster* dadurch altern, daß sie die Weibchen sofort nach der Begattung durch bestrahlte Männchen für 12 Tage bei 15° auf hefefreiem Futter hielten. Sie fanden für den durch ihre Methode erfaßbaren Bereich keinen Unterschied gegenüber der Dislokationsrate bei sofortiger Verwendung der bestrahlten Spermien. Wir stellten den gleichen Versuch mit *D. hydei* in der Weise an, daß die Weibchen einmal sofort nach erfolgter Begattung mit bestrahlten Männchen, ein anderes Mal erst nach Beginn der Ablage befruchteter Eier, für 35 bzw. 28 Tage in eine Temperatur von 8—10° verbracht wurden. In allen Versuchen kam es während dieser Zeit der Abkühlung zu keiner Eiablage, nach Rückführung in normale Zuchttemperatur wurden jedoch in allen Fällen nur große Mengen von unbefruchteten Eiern abgelegt. Es gelingt bei *Drosophila hydei* auch bei niederen Temperaturen nicht, die Spermien in den weiblichen Geschlechtswegen so lange Zeit am Leben zu erhalten.

Der Versuch VI zeigt die Dislokationsrate, die durch die Bestrahlung der unbefruchteten Eier erzeugt wird. Die geringe Ausbeute dürfte mit dem Zustand des Oocytenkerns zusammenhängen, der eine ähnliche Struktur hat wie in den Spermatocyten. Außerdem muß man noch mit einem gewissen Verlust an Dislokationen durch die Meiose rechnen, die erst nach dem Eindringen des Spermiums stattfindet. KANELLIS und RADU (1943) fanden, daß bei Bestrahlung von Eiern von *D. melanogaster* in dem durch ihre Methode erfaßten Bereich nur etwa 1/100 der Dislokationsrate der reifen Spermien ausgelöst wird. Bei uns ist das Verhältnis etwa 1/10. GLASS (1940) hat unter Verwendung einer genetischen Methode, die die Unterscheidung der durch die Eier und die Spermien übertragenen Dislokationen ermöglichte, die beiden Dislokationsraten auf diese Weise geprüft, daß er nach der Begattung die Weibchen bestrahlte. Er fand einen starken Unterschied zwischen den beiden Raten. Translokationen konnte er für die weiblichen Keimzellen im Gegensatz zu den männlichen überhaupt nicht nachweisen. In unserem Material wurden nach der Bestrahlung der Eier auch Translokationen übertragen.

Die Versuche, in früheren Stadien der Keimbahn Dislokationen auszulösen, sind durch die höhere Empfindlichkeit der Entwicklungsstadien von *Drosophila* gegenüber der Bestrahlung erschwert. Die nach einer Bestrahlung mit 4800 r überlebenden Embryonen in 1—23 Std alten Eiern entwickeln sich etwas verzögert bis zur 2. Häutung, um dann restlos abzusterben. Nach einer Bestrahlung solcher Embryonen mit 2400 r geht die Entwicklung bis zur Puppe ungestört vor sich, doch

schlüpfen die äußerlich voll entwickelten Imagines nicht. Nach Bestrahlung von jungen Larven vor der 2. Häutung mit 2400 r geht die Entwicklung normal vor sich und die meisten Puppen schlüpfen. Die Imagines zeigen fast ausnahmslos Mißbildungen an den Flügeln, sind aber fruchtbar. Weibchen und Männchen aus diesen Versuchen wurden mit unbestrahlten Partnern gepaart. In den beiden Kreuzungsrichtungen wurden je 73 F_1-Larven präpariert, jedoch keine Dislokationen gefunden. Bestrahlt man verpuppungsreife Larven mit 4800 r, so bleiben alle Imagines in den Puppen stecken. Nach Bestrahlung mit 2400 r schlüpfen die meisten Fliegen, zeigen zum größten Teil Flügelmißbildungen und sind fruchtbar. Bei Verwendung der als Larven bestrahlten Weibchen wurde in 75 Präparaten keine Dislokation gefunden, bei Verwendung der als Larven bestrahlten Männchen in 80 Präparaten 3 Translokationen. Nach Bestrahlung von 2—3 Tage alten Puppen mit 4800 r konnten die Imagines nicht schlüpfen. Bei Verwendung von 5—6 Tage alten Puppen wird durch die gleiche Dosis das Schlüpfen nicht behindert. Auch hier zeigen fast alle Fliegen Mißbildungen an den Flügeln. Bei Verwendung der als Puppen bestrahlten Weibchen wurde unter 84 Präparaten 1 Translokation gefunden, bei Verwendung der als Puppen bestrahlten Männchen in 73 Präparaten keine Dislokation.

Die Art der Entwicklungsstörungen, die nach der Bestrahlung von Embryonen, Larven und Puppen auftreten, machen es wahrscheinlich, daß die die Entwicklung regelnden innersekretorischen Gewebe besonders strahlenempfindlich sind. Eine quantitative Auswertung der Resultate ist nicht möglich. Sie zeigen jedenfalls, daß auch in den Spermatogonien oder Spermatocyten reifer Larven und in den Oocyten oder Oogonien von Puppen Dislokationen ausgelöst werden können, die die Meiose passieren können und in die Geschlechtszellen gelangen. Die Dislokationsrate ist, dem Zustand der Kerne in den bestrahlten Organen entsprechend, eine sehr geringe. Ob durch die Auslösung einer Dislokation in einem sehr frühen Stadium der Keimbahn eine ganze Gruppe von Gameten mit der gleichen Dislokation erzeugt werden kann, konnte an unserem Material nicht entschieden werden.

Um zu prüfen, inwieweit die strahleninduzierten Dislokationen sich unter den gewöhnlichen Kulturbedingungen in einem Stamm erhalten, wurde aus dem oben besprochenen Versuch I eine Kultur abgezweigt. Diese wurde in der Weise fortgeführt, daß stets die ersten 20 Imagines zur Beschickung eines neuen Glases verwendet wurden. Die Larven der F_4-Generation nach der Bestrahlung zeigen nur mehr eine Dislokationsrate von $4{,}0 \pm 2{,}7\%$ gegenüber $27{,}8 \pm 4{,}6\%$ der F_1-Generation. Es ist anzunehmen, daß abgesehen von den bei der Meiose eintretenden Verlusten an Dislokationen auch eine negative Selektions-

wirkung der Erhaltung der Dislokationen in der künstlichen Population entgegenwirkt.

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Zusammenfassung.

Die durch Untersuchung der Riesenchromosomen der F_1-Larven erfaßbaren, durch Röntgenstrahlen erzeugten, groben Chromosomendislokationen von *Drosophila hydei* zeigen nach Bestrahlung von reifen Spermien mit 4800 r eine Rate von $27{,}8 \pm 4{,}6\%$, nach Bestrahlung von Spermatocyten mit der gleichen Dosis eine Rate von $0{,}7 \pm 0{,}8\%$, bzw. $1{,}0 \pm 1{,}0\%$. In unbefruchteten Eiern wird durch die gleiche Dosis eine übertragbare Rate von $4{,}5 \pm 2{,}3\%$ erzeugt. Die reifen Spermien bleiben in den männlichen Geschlechtswegen auch dann, wenn kein Verbrauch erfolgt, nur kurze Zeit funktionsfähig und werden ständig durch neugebildete ersetzt. Dieser Prozeß läßt sich auch durch stärkere Abkühlung nicht wesentlich verlangsamen. Durch Bestrahlung von erwachsenen Larven und von Puppen lassen sich auch in den früheren Stadien der Keimbahn Dislokationen auslösen, die durch die Geschlechtszellen übertragen werden.

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THE EVOLUTION OF HETEROCHROMATIN IN THE GENUS *TRIBOLIUM (TENEBRIONIDAE: COLEOPTERA)**.

By

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With 8 figures in the text.

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Introduction.

In their cyclical properties of condensation and stainability, euchromatic chromosomes or chromosome regions are strictly coordinated with the cycle of reproduction. Heterochromatic chromosomes, on the other hand, may be out of step either by being more or by being less deeply stained; that is, they may be either positively or negatively heteropycnotic, or even both at different times in one and the same division cycle. The concept that there are different types of heterochromatin has been held by several workers and WHITE (1951) expresses it aptly when he says "Most probably there are many varieties of heterochromatin and euchromatin, constituting a continuous 'spectrum', to the two ends of which the terms heterochromatin and euchromatin are somewhat arbitrarily applied".

DARLINGTON and LA COUR (1938, 1940, and 1941) and CALLAN (1942) have shown that the chromosomes of certain organisms can be induced to stain differentially following subjection to freezing temperatures. Regions that appear euchromatic under normal conditions are thereby made to show heteropycnosis: they have been identified with the prochromosomes of the resting stage.

Experimental work with *Drosophila* led MULLER and PAINTER (1932) to conclude, in agreement with HEITZ (1933 and 1935), that heterochromatin is more or less inert, for it was found that deletions and duplications of it were, in general, much less harmful than comparable changes involving euchromatin. This was already indicated some 25 years earlier by STEVENS' (1908) observations that within populations of certain species of beetles many individuals had from one to four chromosomes over and above the normal complement. Since such supernumerary chromosomes are dispensable for the individual but are

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retained in populations of, for example, bed bugs and maize, DARLINGTON and UPCOTT (1941) have regarded them as sub-inert — sub-inert in that they presumably contribute something to the well-being of the population as a whole.

Supernumerary chromosomes are largely heteropycnotic and thus add weight to the parallel drawn between heterochromatin and genic inertness: they are assumed to carry few, if any, genes having major effects. Similarly the heteropycnosis of sex chromosomes is accounted for by assuming that they carry mainly sex-determining genes, for very few sex-linked genes have so far been discovered even in mammals (PONTECORVO, 1943). The bulk of the evidence to date therefore points to heterochromatin as being largely inert, but that the correlation is by no means complete has been convincingly demonstrated by WHITE's work on gall-midges. He finds both heterochromatic and euchromatic groups of chromosomes in their germ cells but only the heterochromatic set in somatic cells — obviously these can not be completely inert (WHITE, 1950).

MULLER (1918) first formulated the idea that heterochromatin owes its origin to progressive degeneration of euchromatin, such as might have occurred following reduction or suppression of crossing over in the sex chromosomes of the heterogametic sex. But proof of such a transformation remains wanting (BARIGOZZI, 1950; LA COUR, 1951). Moreover, since there is no provision made in MULLER's theory to embrace autosomal heterochromatin, WHITE (1945) has speculated that in some *Orthoptera* it may have had its origin in reduplication of parts of the X chromosome.

Mention should be made of some additional properties, chemical, physical, and genetic, that have been attributed to heterochromatin. Following the pioneer work of CASPERSSON and his colleagues (CASPERSSON, 1939; CASPERSSON et al., 1935) on ultra-violet absorption spectroscopy, DARLINGTON (1942) concluded that heterochromatin plays a special part in the synthesis of desoxyribose nucleic acid. If this is so, it makes understandable why wholly (?) inert supernumeraries have been retained in those groups of organisms in which they occur. Such a chemical function is in accord with CASPERSSON and SCHULTZ' (1938) prior claim that the oocytes of XXY *Drosophila* contain larger amounts of nucleic acid than those of XX flies, and with BRIDGES' (1942) contention that the salivary gland chromosomes of the former stain more intensely than usual. CALLAN (1948), however, has disputed the former claim, and the latter awaits verification.

DARLINGTON and LA COUR (1940) and CALLAN (1942) believe that cold-induced heteropycnosis is simply a matter of nucleic acid content, being independent of degree of spiralization. This view has been effecti-

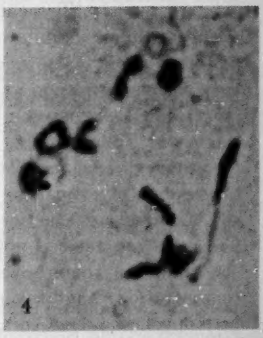
vely disproved by WILSON and BOOTHROYD (1944), employing the same method and plant species as the former workers but reinforced by exact measurements.

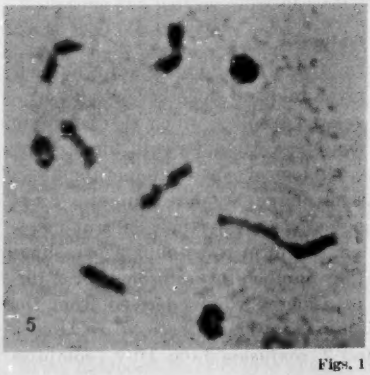
MATHER (1943 and 1944) considers heterochromatin to be the seat of a large number of genes whose individual effects are minute but cumulative. He terms them polygenes in contrast to the major genes which produce large-scale effects. A somewhat parallel stand is taken by PONTECORVO (1944) who regards each heterochromatic segment as being built up of a large number of very similar, if not identical, genes, whereby a condition is reached in which its genetic effects are more or less non-specific.

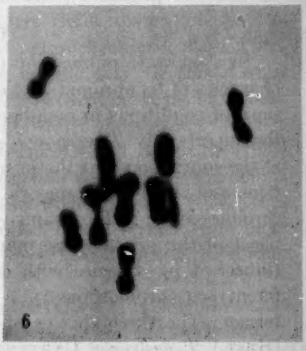
This brief review will serve particularly to underline why CASPERSSON (1947) has felt it necessary to propose the term 'nucleolar-associated chromatin'. In his words, it is intended "to avoid the name heterochromatin, as the cytological and genetical definition of the latter in ordinary nuclei still seems to be very difficult and to some extent a matter of choice". SCHRADER and LEUCHTENBERGER (1950) have briefly considered what is known concerning the relationship of heterochromatin to nucleolus formation and have pointed out that in the final analysis the use of CASPERSSON's term would call for a redefinition of heterochromatin. To circumvent this they advocate that the nucleolus-forming chromatin receive the distinct name "plasmochromatin". The results of the present investigation will likewise go far to show that, taken literally, CASPERSSON's is not a particularly apt term: heterochromatin in *Tribolium confusum* is not of necessity 'nucleolar-associated'. They will also show that heteropycnosis is not merely a matter of nucleic acid concentration; that several kinds of heterochromatin, or degrees of heteropycnosis, can coexist during male meiosis; and that heterochromatin is a phylogenetic derivative of euchromatin.

Observations.

In a separate publication (SMITH, 1952a), the cytology of a number of species of tenebrionid beetles is given in detail, together with descriptions of conditions in members of related families, which also belong in the superfamily *Tenebrionoidea*. Reasons are put forward for arriving at the conclusion that the primitive cytological constitution of the *Tenebrionidae* is 18 autosomes, a relatively large X, and a minute Y sex chromosome (y), the X and y being associated by their ends at metaphase of the primary spermatocytes in a form resembling a parachute (subscript p). Species with deviating numbers of autosomes or a different sex-chromosome type are considered to be derived from the former. The three species of *Tribolium* considered here, *T. castaneum* (HBST.), *T. destructor* UYTTENB., and *T. confusum* JACQ. DUV., comprise







Figs. 1–6.

two numerical classes with the different types of sex chromosomes that characterize the primitive and derivative categories.

T. castaneum has 20 chromosomes in both sexes. In male gonial metaphases one chromosome is minute and spherical and is lacking from those of the female: it is therefore the y chromosome. The X is one of the larger chromosomes, but it cannot be distinguished from the autosomes in gonial divisions. The remaining two species have two fewer chromosomes in both sexes. In spermatogonial metaphases of *T. confusum*, one chromosome, the largest of the complement, is J-shaped and its longer arm is usually visibly split into its two component chromatids. Another member appears to be telocentric and is likewise visibly split: it is identical morphologically to the longer arm of the J-shaped chromosome. In oogonia there are two chromosomes corresponding to the largest chromosome of the male and none corresponding to the telocentric one. The former is therefore the X and the latter the Y. *T. destructor* has one particularly large chromosome in spermatogonial metaphases morphologically like the X of *T. confusum* and a much smaller spherical Y with a diameter roughly equivalent to the cross-section of an autosome, i.e., it is considerably broader than the minute y chromosome of *T. castaneum*.

The sex chromosomes show neither positive nor negative heteropycnosis during gonial divisions; they are open to differentiation from the autosomes only when they differ markedly in size or shape or when they are resolvable into their constituent chromatids. At pachytene, however, they invariably show differential contraction in males, either totally or in part. In *T. castaneum* the paired X and y are condensed, deeply staining, and associated with the nucleolus; in *T. confusum* the XY bivalent, likewise joined to the nucleolus, consists of a short, condensed, heavily stained segment and a long, diffusely stained portion which is indistinguishable from the ordinary euchromatic elements (Fig. 1). Similar conditions probably obtain in *T. destructor*, but additional material is required before this can be stated categorically.

During diakinesis in *T. castaneum* all the bivalents, with the exception of the Xy, stain with equal intensity; at first the Xy bivalent is more deeply stained, but by prometaphase it does not differ perceptibly.

Fig. 1—6. Photomicrographs of primary spermatocyte stages in *Tribolium confusum*; all from Feulgen—light-green 'squash' preparations — × *ca* 4,000.

Figs. 1 to 3. Pachytene showing the nucleolus associated with the neo-XY bivalent situated at 11 o'clock in 1 and 3 — the dark mass above and to the left of the ring-like nucleolus in 3 is caused by extraneous matter; in 2 the centric blocks of bivalents B and D are fused.

Figs. 4 and 5. Mid and later diakinesis showing terminal location of chiasmata, negative heteropycnosis of Y, and relic of Y centric half-block.

Fig. 6. First metaphase: the negatively heteropycnotic Y is longer than the whole X chromosome.

The XY bivalent of *T. confusum* differs in stainability from the autosomal pairs during diakinesis (Figs. 4 and 5): one segment, with a cross-section equivalent to that of the autosomal bivalents, is equivalent also in depth of staining; the other, *longer* segment is noticeably thinner and stains to a much lesser degree, except that it is terminated at its distal end by a dark staining knob. Conditions in *T. destructor* have not been ascertained, due to a lack of satisfactory material.

At metaphase the Xy_p bivalent of *T. castaneum* is possibly somewhat more lightly stained than the autosomal elements (but due to its small size this is difficult to determine with assurance) and lies, at first, just off the equatorial plate, although always orientated on the spindle, with the y nearer the plate than the X. The X and Y in *T. destructor* form a strikingly heteromorphic bivalent at metaphase, which stains with the same intensity as the autosomal bivalents. It fails to show the delayed coordination characteristic of the Xy_p bivalent of *T. castaneum*. The X and Y chromosomes of *T. confusum* also form a heteromorphic bivalent at metaphase (Fig. 6). When stretched on the spindle the X is J-shaped, the Y rod-shaped and invariably joined to the longer arm of the former. The whole of the X chromosome stains with an intensity equal to that of the autosomes, but the Y is relatively pale and of reduced diameter: it alone is negatively heteropycnotic. Of the three components of the sex chromosomes, as seen in spermatogonial metaphases, it is therefore without doubt those of equal length, the telocentric Y and the longer arm of the X, that are united terminally at the first spermatocyte metaphase: they are the pairing arms. The remaining component, the shorter arm of the X, must be the differential arm: a relic of the X chromosome of an Xy_p sex-determining system. There are thus three major components in the XY bivalent of *T. confusum* which may be differentiated in accordance with their reaction to Feulgen's leuco-basic fuchsin: the differential arm of the X — positively heteropycnotic at pachytene but indistinguishable from the autosomes at metaphase; the pairing arm of the X — euchromatic at pachytene and also at metaphase; and the Y chromosome — indistinguishable from euchromatin at pachytene but negatively heteropycnotic at metaphase. Thus, in the terminology of ÖSTERGREN (1950), the differential arm of X and the long distal part of Y are "isopycnotic" at metaphase and pachytene respectively.

At pachytene in *T. confusum* males there are strongly contrasted regional differences in the degree of contraction and stainability of the autosomes also (Figs. 1 to 3). On each side of a constriction, which doubtless marks the position of the centromere, pronounced localized thickenings occur, deeply stained and sharply differentiated from relatively faintly staining and typically paired pachytene threads, which extend from one or both of them. In thus showing heteropycnosis, these

condensed masses render the differentiation of the XY pair from the autosomal pairs at first less certain, for, as will be seen, the XY bivalent is structurally similar. The centric blocks, as they are designated herein, stain solidly and as a consequence show no evidence of having a chromomeric structure; the euchromatic segments, on the other hand, have a distinct beaded structure indicative of chromomeres.

In any one pachytene nucleus there are, then, invariably nine heteropycnotic dumbbell-like blocks composed of two equal-sized, approximately spherical half-blocks, one on each side of the centromere (see particularly Fig. 3). The centric blocks differ somewhat in size but to such a slight degree that they might well be considered as essentially similar. Their dimensions are approximately one and one quarter microns in length and about half as wide. In contrast to the almost uniform size of the half-blocks, there is considerable range in the size of the euchromatic segments attached to them. Three bivalents (*G*, *H*, and *X* in Fig. 7) have such segments on only one side of the block, so that one arm of each consists solely of a completely heteropycnotic half-block. The remaining six have the euchromatic segments of their shorter arms ranging from 1.6 to about five times the length of a half-block. The longer arms have considerably longer euchromatic components and are always longer than the longest of the short arms: they range between 6.6 and 15.4 times the length of a half-block (Table I), that is, between 4.1 and 9.6 microns.

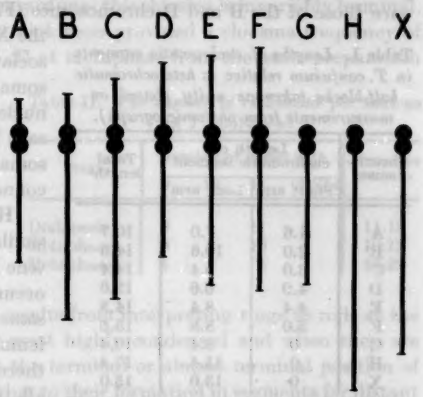


Fig. 7. The nine pachytene bivalents of male *Tribolium confusum* based on a photomicrograph of a flattened preparation. The centric blocks are represented as two spheres separated by the centric contriction; bivalents G, H, and X (the sex-determining one) each have only one euchromatic segment.

The XY bivalent identifies itself at pachytene by its association with the nucleolus (Figs. 1 and 3). Its nucleolar-attached arm is devoid of a euchromatic segment, and is no longer than the half-block that constitutes the proximal end of the other arm. Owing to the small size of the half-blocks, little can be determined concerning their detailed structure, except for occasional indications of longitudinal doubleness within that of the non-nucleolar one. Similar indications of a bipartite structure are

sometimes observable in the half-blocks of the autosomal bivalents, such as would be expected from the pairing of homologous chromosomes. It is implied in the description of the XY half-blocks that the nucleolar arm is a single, unpaired entity, the only one in the complete set of 18. It has not proved possible to secure observational evidence against this interpretation, which on *a priori* grounds seems most logical.

Adhesions of heteropycnotic segments were rarely encountered. Once the centric blocks of the E and F chromosomes were so involved and once those of the B and D chromosomes (Fig. 2). The nucleolar arm of the X was never seen directly associated with the blocks on autosomal bivalents, although the nucleolus itself was occasionally seen lying close enough to autosomal blocks to suggest a possible connection between them.

Table I. *Lengths of euchromatic segments in T. confusum relative to heterochromatic half-blocks taken as unity (based on measurements from photomicrograph).*

Chromosome	Length of euchromatic segment		Total length
	Short arm	Long arm	
A	1.6	7.0	10.7
B	2.0	10.6	14.6
C	3.0	9.4	14.4
D	4.0	6.6	12.6
E	4.4	8.4	14.8
F	5.0	8.8	15.8
G	0	8.4	10.4
H	0	15.4	17.4
X	0	13.0	15.0

Heteropycnotic centric blocks similar to those seen at pachytene in the male of *T. confusum* occur also in the male of *T. castaneum* and, moreover, in the female of *T. destructor*. They are therefore a property common to all three species and, unlike the heteropycnosis of the sex chromosomes, are independent of the male genotype. A similar situation has been reported by HACKMAN (1948) who observed heteropycnotic blocks at pre-leptotene in the female as well as the male of certain spiders. In very young oocytes, or possibly nurse cells, of *T. destructor* the number of blocks is 18, thereby showing that their formation precedes chromosome pairing, as it should if they are identical with prochromosomes. Moreover, the occurrence of 18 blocks in females having 18 as the diploid number strongly suggests that the X chromosomes, although essentially non-heteropycnotic in females, are structurally similar to the autosomes in their possession of centric blocks.

Chiasmata have never been directly observed by resolution into their component chromatids in the three species of *Tribolium* under consideration. But by analogy with conditions in *Zopherus haldemani* SALLÉ (SMITH, 1952a), as well as those depicted by GUÉNIN (1950) in *Diaperis boleti* L., it seems plausible to assume that the terminal unions characteristic of bivalents at metaphase in *Tribolium* are the result of preceding interchanges. With the reservation that their existence has not been proved herein, the term chiasmata will therefore be used to designate the unions between the component chromosomes of bivalents.

At the earliest stage of spermatogenesis at which chiasmata have been observed, they are almost always terminal in both ring- and rod-shaped bivalents. Out of a total of 167 chiasmata counted only four were subterminal, and, of these, three were in different rings and one in a rod. The number of ring bivalents at diakinesis ranges from one to three per nucleus and at metaphase from zero to three. The maximum number of chiasmata in a bivalent is two for rings and one in the case of rods, i.e., one per arm. The sex-determining bivalent, being J-shaped, always has a chiasma frequency of one: this chiasma is invariably terminal.

A sample of 15 nuclei at diakinesis provided a chiasma frequency of 11.13. A further sample of 25 at metaphase from the same preparation gave a value of 10.72, and 13 metaphases from a second individual a value of 10.23 (Table II).

Table II. *The number of chiasmata per nucleus in T. confusum.*

Stage	Number of nuclei with				Mean per nucleus
	9	10	11	12 Xta	
Diakinesis .	0	1	11	3	11.13
Metaphase .	0	8	16	1	10.72
Metaphase .	4	3	5	1	10.23

These frequencies suggest that it is possible for chiasmata to be lost before metaphase, but more probably the apparent decrease in frequency at metaphase results from interpreting rings as rods at the time that the bivalents are most highly condensed and when they are viewed from the side. That the terminal or almost terminal position of chiasmata is most probably due to their formation in segments far distant from the centromere is strongly suggested by their positions at early diakinesis. There, as already stated, they occur in terminal or almost terminal positions at a time when there is no evident 'repulsion' between the centromeres (see particularly Fig. 4). In other words, their distal localization at diakinesis in rod bivalents, at any rate, is not due to terminalization. The absence at metaphase of subterminal chiasmata in ring bivalents might, however, be due to this factor.

Omitting the XY bivalent because one arm of the X chromosome constitutes a differential segment, the maximum potential number of ring bivalents is eight. Since this maximum is never even remotely approached, it is clear that the heterochromatic half-blocks are precluded from chiasma formation. Rings are apparently formed only by those bivalents that have a minimum of size difference between the two arms: most probably chromosome types D, E, and F (Fig. 7). Moreover, since more than three rings were never observed, the minimum length of euchromatic segment necessary for chiasma formation is evidently close to four times the length of a centric half-block.

Presumably as a result of the terminal location of all chiasmata at metaphase, anaphase disjunction usually proceeds without undue

stretching. In plants of two species of *Trillium* which were cold-treated, DARLINGTON and LA COUR (1940) have shown that certain of the understained segments exhibit difficulty in separating at mitotic anaphase and may eventually suffer breakage. These "reproductive errors" are attributed by them to deficiencies in nucleic acid. WILSON and BOOTHROYD (1944) have suggested that only one species of *Trillium*, *T. stylosum*, was examined by DARLINGTON and LA COUR and that the "faulty anaphase separation is typical of only this one species, or of only their stock of it, and may have a cause quite distinct from differentiation". It therefore seemed worthwhile to secure an estimate of the ease of separation of the sex chromosomes relative to the autosomes in *T. confusum* so as to determine whether the former were in any way handicapped by the negative heteropycnosis of the Y. Ten nuclei between full metaphase and early anaphase were selected and the distance between the extremities of all bivalents measured from camera lucida drawings.

A characteristic feature of *Tribolium* and many other beetles is the failure of the chromosomes to show a flexion at the first meiotic metaphase indicative of the position of the centromere: this is revealed occasionally in *T. confusum* and regularly in some other species only when pronounced stretching of the bivalents precedes anaphase disjunction (SMITH, 1952a). Consequently in *Tribolium* difficulty is experienced in determining whether a particular chromosome is essentially one-armed, such as types G and H, or two-armed, such for example as type F. Since the distance between the extremities of a bivalent of the latter type is much greater when only one rather than two chiasmata are present, ring bivalents (italicized in Table III) and potential ring-forming bivalents (those in the last column) have been omitted in determining the mean length of the autosomes. Taking the length of the XY bivalent as a chronological criterion, there appears to be a general tendency for

Table III. *The overall lengths of the nine bivalents in each of ten nuclei in T. confusum between full metaphase and early anaphase.*

Length of XY	Lengths of eight autosomal bivalents *								Mean of first 5 autosomes	Ratio A/XY
10	5	5	5	5	7	6	6	7	5.4	5.4
10	5	5	5	5	6	*4*	*4*	6	5.2	5.2
10	5	5	5	5	6	*4*	*4*	6	5.2	5.2
11	5	5	5	6	9	6	6	8	6.0	5.5
11	6	6	7	7	8	*4*	*5*	7	6.8	6.2
12	5	5	5	5	9	*4*	*4*	6	5.8	4.8
12	5	5	5	6	7	6	7	7	5.6	4.7
13	5	6	6	6	10	*4*	7	7	6.6	5.1
13	5	5	6	6	7	*4*	*4*	6	5.8	4.5
14	5	5	6	7	8	*4*	*4*	*5*	6.2	4.4

* numbers italicized are lengths of ring bivalents.

all bivalents to elongate with the approach of anaphase, a tendency which is perhaps somewhat more marked in the case of the XY pair. However, since the actual separation is rather strictly coordinated for all elements of a nucleus, the disproportion between the XY and the autosomal bivalents may possibly be due more to the greater elasticity of the thinner and paler Y than to a stronger tendency for the X and Y chromosomes to adhere.

To test this contingency measurements were made of the X and Y components in 10 nuclei from an individual in which there was a wide range in the degree of the pre-anaphase stretch. Two nuclei were classed as being at early, four at mid, and four at late metaphase. The average distances between the terminal chiasma joining the X and Y chromosomes and their ends (*not* their centromeres) are given in microns in Table IV.

Table IV. *Relative degree of stretching of the X and Y chromosomes through metaphase in T. confusum: Lengths expressed in microns.*

Stage	Length of X	Length of Y	Ratio X to Y
Early metaphase	2.5	3.5	1:1.40
Mid metaphase	3.5	4.3	1:1.23
Late metaphase	5.0	6.6	1:1.32

These values show that the relatively greater stretching of the XY bivalent is attributable to both the X and Y components and not to the latter disproportionately.

Owing to the sex-determining bivalent being identifiable with certainty at all stages of the first meiotic division and particularly because it is composed of positively heteropycnotic, negatively heteropycnotic, as well as euchromatic segments, it has proved possible to analyze the process of contraction of these different elements with some degree of accuracy. In view of the general lack of agreement as to the relationship existing between heteropycnosis, whether positive or negative, and contraction, this is at once worthwhile for although at pachytene the length of the X chromosome exceeds that of the Y chromosome by

Table V. *Relative lengths of X and Y chromosome segments in T. confusum at various stages of meiosis and at spermatogonial metaphase.*

Stage	X nuc. arm	X non-nuc. half-block	X euch. segment	Y excl. half-block	Y half-block
Pachytene	*1*	*1*	*13.0*	*13.0*	*1*
Early diakinesis	2	1	4.0	6.5	1
Mid diakinesis	2	1	3.0	7.0	1
Late diakinesis	2	1	2.0	6.0	1
Early metaphase	2	1	1.0	4.5	1
Mid metaphase	2	1	2.5	6.0	1
Late metaphase	2.5	1	4.5	10.0	1
Spermatogonial metaphase	2	1	2.0	2.0	1

the length of its nucleolar-attached arm, it is obvious even at the most casual glance that the relative lengths of the two chromosomes are reversed by metaphase. Clearly there is a differential in contraction.

The relative lengths of the various segments comprising the X and Y chromosomes have been determined (Table V) and are represented diagrammatically in Fig. 8. The lengths, expressed in relation to the length of a centric half-block (taken as unity in the table, but actually approximating 0.6 μ), at pachytene, early, mid, and late diakinesis have been determined from photomicrographs; those for early, mid, and late metaphase have been measured from camera lucida drawings.

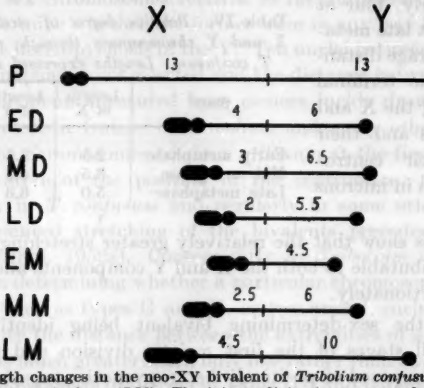


Fig. 8. The length changes in the neo-XY bivalent of *Tribolium confusum* between pachytene (*P*) and late metaphase (*LM*). The intermediate stages are early (*ED*), mid (*MD*), and late (*LD*) diakinesis, early (*EM*), and mid (*MM*) metaphase.

The values given in italics in Table V are based on regional measurements; the remainder are derived from overall measurements of the X and Y chromosomes on the provisional assumption that two of the three centric half-blocks invariably constitute one unit of length whereas the third, the nucleolar arm, doubles its length by early diakinesis (see Discussion).

Discussion.

The formula 9AA + Xy_p being accepted as basic for the *Tenebrionidae* (SMITH, 1951 and 1952a), it follows that the differences between *T. castaneum*, on the one hand, and *T. destructor* and *T. confusum*, on the other, both in chromosome number and sex-chromosome morphology, have resulted from autosomal-sex chromosome translocation. Comparable neo-XY sex-determining mechanisms have long been known in *Orthoptera* and have more recently been discovered in Coleoptera (ASANA, MAKINO, and NIIYAMA, 1942; YOSIDA, 1949b; SMITH, 1949).

The neo-X and neo-Y of *T. confusum* have been identified as such because of their numerical relationships with the two sexes. The larger chromosome being a composite neo-X with its longer arm homologous to the telocentric chromosome, then the formula of the male is AA + XO and that of the female AA + XX. In this interpretation, which alone fits observation and theoretical expectation, there is engendered the anomaly of one of a pair of previously autosomal elements being heterochromatic in the sex heterozygote, although the other is euchromatic. In conformity with other cases (SMITH, 1952a), one would expect the free, differential arm to be heteropycnotic in first metaphases, but this is obviously not the case. There is therefore the dual anomaly of the component that should be non-heteropycnotic at metaphase being heteropycnotic and the component that should be heteropycnotic being in fact non-heteropycnotic. The latter peculiarity, although at variance with the conditions of heteropycnosis established for most Xy_p species, is in itself perhaps of no particular significance, for a similar behaviour has been noted in most neo-XY *Coleoptera*. It is the negative heteropycnosis of the neo-Y that calls for further consideration.

KOLLER (1938) and PONTECORVO (1943) have shown in two species of Hamsters, *Cricetus auratus* and *Cricetulus griseus*, that the heterochromatic X chromosome is composed of two distinct regions differing from each other and from the euchromatic autosomes in their "nucleic acid cycle". The pairing segment of the X, like the whole of the Y, is positively heteropycnotic during prophase, whereas the differential segment of the X is negatively heteropycnotic at metaphase. The phenomenon is thus similar to that seen in *T. confusum*, except that the stainability of the X differential segment and the Y chromosome in the two is reversed. MAKINO (1951) similarly reports that there are two different reactions in the stainability and degree of contraction of the X and Y chromosomes of certain field mice of the genus *Apodemus*. Therein the situation is somewhat different, however, in that both the X and the Y have positively heteropycnotic regions that persist as such from pachytene until metaphase and, moreover, regions that are non-heteropycnotic at pachytene yet exhibit negative heteropycnosis at metaphase. Following HEITZ' original (1929 and 1932) definition, and contrary to PONTECORVO, MAKINO refers to these as heterochromatin and euchromatin respectively: this is unfortunately in conflict with more recent and logical terminology and, as will be seen particularly later, conflicts with the situation as it exists in *T. confusum*.

From a survey of the literature, PONTECORVO (1943) is inclined to believe that the presence of differential segments of the type found in *Cricetulus* is a widespread feature of mammalian sex chromosomes, and he discusses them from the point of view of their genetical properties.

In view of the fact that, with the exception of man and possibly the "tortoiseshell" cat, no mammal is known to carry genes that are either completely or incompletely linked to the sex-determining genes [1], PONTECORVO is led to conclude that, with the exception of those of man, the sex chromosomes in the group as a whole carry essentially nothing other than sex genes. Furthermore, he considers that "...the sex differentiating function comes to be based on the balance between female sex-genes, in the differential segment of X, and male sex-genes, in the pairing segments of X and Y or in the autosomes". Similarly, sex-linked genes giving well-defined alternative phenotypes are almost unknown in beetles (see FERWERDA, 1928, cited by GUÉNIN, 1950).

In Xy_p species, such as *Tenebrio molitor* which was studied genetically by FERWERDA, completely sex-linked genes must be carried in the differential segments which occur there on each side of the centromere, with chiasmata, if any are formed, being restricted to the extreme distal ends (SMITH, 1949 and 1951). The situation with regard to heterochromatin and chiasma localization is different in *T. confusum* from that claimed by PONTECORVO for mammals. In the latter, chiasma formation is said to be confined to the regions that are positively heteropycnotic at pachytene, yet, by analogy with the proximal blocks in *T. confusum* it is from precisely such positively heteropycnotic segments that chiasma formation should be precluded. Both MATTHEY (1947) and MAKINO (1951) find unacceptable the view first expressed by KOLLER and DARLINGTON (1934) that chiasmata play a special role in holding the sex chromosomes of the rat in association between pachytene and metaphase. In view of the apparent absence of euchromatic segments from the sex chromosomes of Xy species of beetles, MATTHEY and MAKINO would doubtless be sceptical of any interpretation which held chiasmata as responsible for metaphase association of the sex chromosomes in them. However, the centric blocks in *T. confusum* and all other species that are known to possess them fail to retain their pachytene association at all later stages. Furthermore, certain species of Xy beetles regularly have the parachute type of metaphase association replaced by a single point of contact (see ASANA, MAKINO, and NIIYAMA, 1942; YOSIDA, 1944 and 1949a, among more recent papers). It may well be, then, that very short euchromatic segments are present (though undetected to date) in which chiasmata are formed and to which they are restricted. However, in beetles at least, chiasma formation in hypothetical segments of such limited length would clearly be governed by principles entirely distinct from those that will later be shown to operate in *T. confusum*. Alternatively, of course, if the segments in which chiasmata occur at meta-

[1] Dr. WHITE informs me that according to Dr. W. L. RUSSELL (unpub.) a sex-linked gene has recently been discovered in the mouse.

phase in such mammals as *Cricetulus* were euchromatic at pachytene and it proved to be the segment that is negatively heteropycnotic at metaphase that was positively so at pachytene, the anomaly would be readily explained.

Evidence has been accumulated (SMITH, 1950 and 1952c) for the occurrence of neo-XY systems in 18 or more species of beetles in the *Carabidae*, *Buprestidae*, *Erotylidae*, *Coccinellidae*, *Lagriidae*, *Melandryidae*, *Chrysomelidae*, and *Curculionidae*, besides those in other *Tenebrionidae*. With the exception only of those of *Tribolium confusum*, the autosomal components of the neo-XY pair invariably stain with equal intensity and yet, with two further exceptions, are of equal size and shape, that is, the neo-Y has a seemingly terminal centromere. The centromere of the neo-Y is clearly subterminal, to the best of my knowledge, only in *Diaperis boleti* L. (GUÉNIN, 1950) and *Luperodes praeustus* MOTSCH. (YOSIDA, 1949b), so that in these species alone it is possible to have a part of the neo-Y restricted in its entirety to the male sex without regard to the position of exchanges between the arms common to the neo-X and neo-Y. Such a segment, being isolated in the male line, could have become the carrier of the male sex-genes and other completely Y-linked genes.

Approximately one-seventh of the species of *Coleoptera* known cytologically lack a Y chromosome in their sex-determining systems (SMITH, 1952c). As a consequence it is to be inferred either that they lack male sex-genes altogether or, more likely, that they have had the male sex-genes or their functions taken over by autosomes (see, however, SMITH, 1952a). It may therefore be safely concluded that differential segments of the Y chromosomes do not play a very fundamental role in the genetics of sex-determination in beetles.

Perhaps the closest analogy to the heteropycnotic centric blocks in the pachytene chromosomes of *Tribolium* is to be found in the tomato (BROWN, 1949; BARTON, 1951; GOTTSCHALK, 1951). BROWN has described the differentiation of the pachytene chromosome arms into relatively broad, deeply-stained regions adjacent to the centromeres ("chromatic zones") and distal, relatively narrower, lightly-stained regions ("achromatic zones"). In nuclei at diakinesis all chiasmata were found to be restricted to the achromatic zones and, in agreement with *Tribolium*, it seems highly improbable that they were ever formed in the proximal chromatic zones. On the basis of careful measurement, BROWN concluded that the achromatic zones, which at pachytene comprise considerably more than half the total chromosome length, contribute only a small portion to the overall length of the chromosomes at late prophase of either meiosis or mitosis. As will be shown this is again in complete agreement with *Tribolium*.

In Table V the lengths of the various segments comprising the X and Y chromosomes of *T. confusum* at different stages of meiosis and at spermatogonial metaphase are expressed relative to the length, *ca.* 0.6 microns, of a centric half-block. In analysing these, the mechanical basis for the observed increase in length of the nucleolar arm will first be considered. At pachytene the highly compact centric half-blocks, whether autosomal or in the sex-determining pair, are all of essentially the same size and measure roughly 0.6 microns both in length and width. The X chromosome half-block on the side across the centromere from the nucleolar arm is obviously formed by the joint coiling of the X and Y chromonemata, whereas that of the nucleolar arm consists of only a single chromonema disposed in a similar spiral. From the fact that the two are of equivalent size, it follows that the length of the chromonema of the nucleolar arm must be about double that of the individual chromonemata jointly forming the half-block of the Y and the non-nucleolar arm. Since the width of the nucleolar arm remains reasonably constant between pachytene and metaphase, it is evident that the 'splitting' of the chromosomes into chromatids by diplotene is causally related to the doubling of the length of the nucleolar arm. That no comparable increase in the length of the non-nucleolar half-block occurs is doubtless because its component homologues then separate to form two distinct half-blocks. It is, of course, not possible to make direct measurements of the length of the half-block of the non-nucleolar arm after pachytene for, like those of the autosomes, it becomes progressively indistinguishable from the euchromatic portion with the approach of metaphase; those blocks associated with such euchromatic segments *appear* to increase in size gradually at the expense of the latter. If, however, it is tentatively assumed that all half-blocks other than that constituting the nucleolar arm remain unchanged in size between pachytene and metaphase, then the contribution made by the euchromatic segments to the ultimate size of the components of a bivalent may be readily estimated, and the validity of the assumption is open to test.

It will be seen that, relative to their lengths at pachytene when they are demonstrably equal, the euchromatic segment of the X chromosome and the negatively heteropycnotic segment of the Y have characteristic contraction factors during the ensuing stages. The former is consistently contracted to a higher degree than the latter, and both reach a maximum compactness by early metaphase. At this time, however, the relatively thin and pale Y chromosome is still some four and one-half times the length of its counterpart in the X. Part of this excess in length of the Y over the X is doubtless to be attributed to a redistribution of the bulk of the Y centric half-block for by early metaphase it is to be distinguished

from the major part of the chromosome only by being somewhat wider. As well as can be judged it appears to be about half as wide as it was at pachytene, so that it might reasonably be considered as contributing about two units to the overall length of the Y chromosome. This being granted, there are five distinct factors operating in the development of the XY bivalent between pachytene and metaphase. These are:

(1) the differential for the euchromatic segment of the X — 13.0:1

(2) that for the major part of the Y, the negatively heteropycnotic segment — 13.0:3.5

(3) the doubling of the length of the positively heteropycnotic nucleolar arm of the X

(4) the similar, but causally different, doubling of the length of the centric half-block of the Y

(5) the uniformity of dimension of, or at least the lack of increase in, the half-block in the non-nucleolar arm of the X chromosome.

The five spiralization coefficients (DARLINGTON and UPCOTT, 1939) are therefore: 13, *ca.* 4, 1, $^1/_2$, and 1, which doubtless would be interpreted by some workers as showing that each segment has a reactivity in nucleic acid synthesis of its own. The results obtained herein go far to support WILSON and BOOTHROYD (1944) in the contention (*contra* DARLINGTON and LA COUR, 1938 and 1941) that heteropycnosis v. non-heteropycnosis is more a matter of relative density of spiralization than one of degree of charging with nucleic acid. On the other hand, WHITE (1940) came to the conclusion "...heteropycnosis is not merely a matter of 'differential contraction' as many workers appear to have supposed; it is due to differential thickening or lateral growth'. Here of course he is considering positive, not negative, heteropycnosis.

Judging by their conformation at metaphase only the first and last coefficients are operative in the contraction of autosomal bivalents. If all the autosomal blocks are of equal size, the differences between the ultimate sizes of the bivalents must reside in the contributions made by their associated euchromatic segments. If the latter likewise contract to one-thirteenth of their pachytene lengths, they would provide a range in size of between $1.25 + 0.66$ and $1.25 + 1.18\,\mu$, or between slightly less than two and slightly less than two and one-half microns, for the smallest and largest autosomal bivalents. That the bivalents appear to range outside these extremes is doubtless due to the lack of strict uniformity already noted among bivalents in the size of their individual centric blocks, and points to the possession by the autosomal euchromatic segments of a spiralization coefficient of 13.0, while at the same time establishing the validity of the assumption that the size of the autosomal blocks remains constant.

In Table IV it was shown that the stretching of the XY bivalent during the course of metaphase is attributable equally to the two chromosomes involved. When, however, the two components of the X, the differential and the pairing arms, are measured separately, it is found (Table V) that their lengthening is disproportional, being from 2:0 to 2.5 units for the former and from 2.0 to 5.5 for the latter. With a simultaneous increase in the Y of from 5.5 to 11.0, it is clear that the homologous arms stretch differentially, X having a factor of $\times 2.75$ and Y one of $\times 2.0$. This is perhaps most readily explained as resulting from the closer coils of the X arm yielding more readily to the strain of anaphase than the already relaxed coils of the Y.

BROWN's (1949) comparative figures for contraction in *Solanum* differ from those obtained herein in showing a moderate decrease in the length of the "chromatic zones". It will be clear from a glance at his Plate I, however, that the degree of linear contraction of the chromatic zones at pachytene, as expressed both in their greater length and their lesser width, is considerably less than is the case in *Tribolium*. Further lengthwise contraction is possible in *Solanum* not only by increase in width, presumably by spiralization, but also by the closing-up of the interchromomeric 'gaps' which are present there. Gaps do not show in the centric blocks in *Tribolium*: presumably the chromomeres are already in contact at pachytene, and the regions are fully spiralized.

In spermatogonial metaphases the Y chromosome and the homologous arm of the X chromosome are of equivalent dimensions, each being about half as long again as the differential arm of the latter. The difference between these proportions as compared with those determined at first meiotic metaphase (Table V) may be attributed to the underspiralized condition of the negatively heteropycnotic Y chromosome at the latter stage. Differential length changes of a similar nature might readily account for the inability of both KOLLER (1938) and PONTECORVO (1943) to demonstrate in the Golden Hamster linear differences at spermatogonial metaphase comparable to those observed there at meiotic metaphase.

The demonstration of five different spiralization coefficients operating in contraction fits well with the generally held belief that there are a number of types of heterochromatin and euchromatin. It is to be noted further that the different spiralization coefficients afford strong support for CALLAN's (1942) view regarding the relation between the presence or absence of chromocentres at early stages and the degree of spiralization at metaphase. *T. confusum*, in fact, serves as a natural experiment in that in one and the same division cycle the condensed centric blocks retain their extreme spiralization, while the unspiralized part of the Y

achieves only limited spiralization. It must, however, be stressed that by introducing the concept of competition for available supplies of nucleic acid, CALLAN's views become open to criticism, for the "heavily charged" heterochromatic blocks of pachytene are visibly just as "heavily charged" at metaphase. It appears that DARLINGTON and LA COUR (1940) may therefore have been correct in concluding that "...nucleic acid seems to have no necessary relation to spiralization", but quite evidently not for the reasons they put forward.

Heterochromatin and euchromatin are visibly differentiated cytologically, at least in *Tribolium*, by their possession of characteristic cycles of spiralization (and perhaps of desoxyribonucleic acid content) which are under the influence of the genotype and the immediate physical environment. That the capacity of heterochromatic chromosomes to pair *inter se* and undergo crossing over is dependent on to what degree they are diffusely distributed at zygotene and pachytene is shown by the behaviour of sex chromosomes and supernumerary chromosomes. Thus, in tetrapoloid spermatocytes of *Orthoptera*, the two condensed X chromosomes come to lie side by side but fail to form chiasmata (WHITE, 1933), although, of course, the two do so regularly in normal oocytes (MCNABB, 1928). The B chromosomes of *Zea mays* are only regionally heteropycnotic at pachytene, however, and chiasma formation is restricted to the portions that behave in a manner similar to euchromatin (MCCLINTOCK, 1933); those of *Anthoxanthum aristatum* (ÖSTERGREN, 1947) are positively heteropycnotic during mitotic prophase, weakly negatively so at mitotic metaphase, and yet at pachytene are completely non-heteropycnotic and pair regularly between themselves. Moreover, where seemingly entire heteropycnotic chromosomes form chiasmata with considerable regularity, as do the supernumerary chromosomes in *Trimerotropis sparsa*, it is found that this property is restricted to the relatively short euchromatic segment that occurs close to the distal end of the chromosomes (WHITE, in press). Finally, in *Tribolium* condensation involves spiralization together with the "packing" of adjacent chromomeres at the expense of the interchromomeric fibres, just as it does in *Orthoptera*. Chiasmata are thereby excluded from the proximal heteropycnotic blocks.

Apparently nowhere in discussions dealing with the physicochemical constitution of euchromatin and heterochromatin is it implied that there are fundamental differences in the nature of the protein fibres that go to make up their interchromomeric fibres. There is, then, presumably no intrinsic barrier to crossing over within and between two segments that are respectively euchromatic and heterochromatic, provided only that they pair with precision at zygotene. Now, the autosomal components of the neo-XY bivalent in *T. confusum* are distinguishable from

the other bivalents of the pachytene nucleus only in being associated through the differential arm of the X with the nucleolus: so far as can be determined optically, there is at that stage a local uniformity of stainability, contraction, and structure common to both the sex chromosomes and the autosomes. The two arms of the neo-XY that are only later identifiable as euchromatic and heterochromatic are therefore precisely paired and in a position to engage in exchanges anywhere along their length. That these exchanges are realized only in a distally restricted zone is shown by their localization there when first observable at early diakinesis. It is also to be inferred from the invariable distribution of euchromatin and heterochromatin between the two at metaphase. It therefore appears that chiasma formation is precluded from the interstitial regions of the paired XY arms, not by their differentiation into euchromatin and heterochromatin, but by a genetically controlled distal localization of chiasma formation common to the sex chromosomes and the autosomes.

MULLER (1918) was originally responsible for the suggestion that heterochromatin has originated from euchromatin by a process of "progressive degeneration", i.e., through successive mutation to inertness of genes in the Y chromosome kept permanently heterozygous by the absence of crossing over. There is admittedly no direct evidence, other than that inferred from such diagnostic properties as differential staining and precocious condensation, that the centric blocks visible in *Tribolium* are genetically inert, or even sub-inert. Their localized position and their remarkable constancy in shape and form indeed point to a reaction determined by the close proximity of the centromere, as envisaged by DARLINGTON (1937, *et seq.*). Nevertheless, they are manifestly excluded from crossing over, so that their constituent genes are permanently linked (as also in the female), but in theory they should be exempt from mutation to inertness. If on the other hand they are now in fact inert, as is suggested by analogy with *Drosophila*, that they have retained their uniformity in size, shape, and position may perhaps be a direct result of the proximity of the centromere through its function in controlling the position in which chiasmata are formed (the differential distance — MATHER, 1937 and 1940). Once the proximal genes have acquired inertness, their subsequent loss would render the more distal, active genes liable to exclusion from crossing over and so lower the recombination index. Such a procedure would clearly result, in the long run, in the complete extinction of genic recombination and, more important, chiasma formation — there would quite obviously be strong selection against such a course. The centric blocks may therefore be regarded as being far from dispensable, regardless of any special function that may be attributed to them in connection with nucleic acid metabolism.

It is of interest to note in this connection that, from an extensive survey of the literature, PONTECORVO (1944) has more recently come to the conclusion that the distinctive cyclical behaviour exhibited by heterochromatin is to be attributed, not to a failure in competition with euchromatin for a limited supply of desoxyribonucleic acid, but to it being less differentiated internally than euchromatin. From this he goes on to draw a parallel between this supposedly greater internal uniformity of structure and the sub-inertness of the genes located in heterochromatin, which has served as a basis for MATHER's (1943 and 1944) concept of the location of polygenes. The observational datum secured at pachytene regarding the structural similarity of the euchromatic and heterochromatic pairing arms of the X and Y chromosomes of *Tribolium* clearly speaks as strongly against PONTECORVO's thesis as it does against an explanation based on a differential in nucleic acid requirement. As a consequence, the correlation he envisages between a lesser internal differentiation of heterochromatin and the limited phenotypical and physiological effect of polygenes is, to say the least, decidedly impaired.

Although heteropycnotic differences confined to the pairing arms of the neo-XY are otherwise unknown in *Coleoptera*, the positively heteropycnotic centric blocks of heterochromatin are of widespread occurrence. They have been found in both the autosomes and the sex chromosomes of a number of species in widely different families and in such other *Tenebrionidae* as *Tenebrio picipes* HBST., *Zopherus haldemani* SALLÉ, and *Upis ceramboides* (L.) (SMITH, 1952a). Should the blocks be in fact inert, it is not improbable that they play an important functional role in the evolution of the karyotype, comparable to that envisaged for the analogous regions in *Drosophila melanogaster* (DUBININ, 1936, cited in BABCOCK, 1947) and *Crepis* (TOBGY, 1943; BABCOCK, 1947), as has recently been pointed out by SMITH (1952a and b).

Whether the genic prohibition against other than distal exchanges, seen to operate so precisely in both the sex chromosomes and the autosomes, is causally related to the differential staining of the neo-Y needs next be considered. Despite the probability that the same limitation was operating prior to the time at which the neo-Y became incorporated into the sex-determining mechanism, and regardless of the fact that the same localization has induced no comparable differentiation in the remaining autosomal chromosomes, it seems probable that the negative heteropycnosis of the major part of the Y is attributable to the restriction of crossing over to its distal extremity. Unlike those of the autosomes, the region of the neo-Y proximal to the first distal chiasma has, since its inclusion in the sex-determining system, been kept permanently restricted to the male sex and thus particularly liable to the progressive

degeneration envisaged by MULLER. Thus the negative heteropycnosis of the neo-Y may be looked upon as a consequence of its evolutionary isolation.

The neo-Y of *T. destructor*, unlike that of *T. confusum*, is much shorter than the autosomal component of the neo-X, both at mitotic and meiotic metaphase. It is similar to the neo-X, however, in cross section. It is clear then that it has suffered extensive deletion: it lacks a negatively heteropycnotic segment. In view of its close relationship to *T. confusum* — HINTON (1948) has put both species close together in the same species-group, whereas *T. castaneum* is placed in a distinct one — it seems most probable that *T. destructor* is primarily differentiated from *T. confusum* cytologically in having lost a considerable segment from its Y chromosome. Since the loss was effected without the extinction of the species, the region must previously have been to a greater or lesser degree inert and presumably at some prior time have shown the allocycly characteristic of the neo-Y of *T. confusum*. This may therefore be taken as further evidence that the negative heteropycnosis of the neo-Y in *T. confusum* denotes genetic inertness.

WHITE at one time (1945, p. 252) hesitated to look upon the neo-XY system in certain races of *Gryllotalpa gryllotalpa* as resulting from autosomal-X translocation because of the heteropycnosis of the neo-Y. He conceived of the Y in these and some other similar forms as possibly being derived from the X chromosome, although admitting that no such case had at that time been established. BARIGOZZI (1950) expresses his opinion in the following words "There is no proof that euchromatin can transform itself into heterochromatin ...", and LA COUR (1951) agrees that it is not known whether heterochromatin can arise from euchromatin or *vice versa*. Recently, KING (1950) has examined the grasshopper *Mermiria intertexta*, a species which in some ways resembles the races of *Gryllotalpa* discussed by WHITE, and has reached the following conclusion: "Judging by heteropycnosis and size, it seems clear that we have here a relatively advanced stage in the development of differential segments in both the neo-Y chromosome, and in that part of the neo-X formerly homologous to the neo-Y." He visualizes the course of events as being as follows: (1) centric fusion of the X and an autosome; (2) the development (or acquisition) of heterochromatin throughout the length of the homologue of the autosome concerned; followed by (3) translocation of a heterochromatic region, possibly from the heteropycnotic homologue, to the autosome attached to the X. By leaving open the question as to how the heterochromatin originated, he therefore fails to establish irrevocably that it has been evolved from euchromatin. The evidence from *T. confusum*, however, appears to be unassailable. The neo-Y is fully negatively heteropycnotic at metaphase, but it pairs at

pachytene, *as well as can be seen*, throughout its length with the autosomal part of the neo-X. The two are therefore homologous and the evolution of heterochromatin from euchromatin is thereby proved.

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Summary.

Tribolium confusum differs from *T. castaneum* in having a neo-XY sex-determining mechanism. The neo-Y is negatively heteropycnotic at metaphase I. At pachytene all bivalents have positively heteropycnotic blocks consisting of approximately spherical half-blocks separated by the centromere. The half-blocks throughout the complement measure about 0,6 μ. Three bivalents, including the XY, have euchromatic segments attached to only one half-block; the remaining six have them attached to each. The bivalents are individually recognisable, and the X is associated with the nucleolus through its purely heterochromatic arm.

Five distinct spiralization coefficients are concerned in the condensation of the XY pair between pachytene and metaphase. Only two are operative in autosomal contraction.

The following conclusions have been reached:

1) Differential reactivity in *Tribolium* is primarily a matter of relative density of spiralization.

2) The centric blocks, being heteropycnotic in both sexes, differ from the heterochromatin of the relic X, which is heteropycnotic in the male only.

3) The capacity of heterochromatin to undergo crossing over is dependent on its specific cycle of spiralization coinciding with that of the euchromatin at zygotene and pachytene. Chiasma formation is therefore totally precluded from the centric blocks. Distal localization of chiasmata is genotypically determined.

4) The centric half-blocks in this way constitute a minimum value for MATHER's differential distance, which, however, is independent of chromosome length.

5) A certain minimum length of euchromatin is required to accommodate chiasmata.

6) That the paired arms of X and Y are indistinguishable at pachytene is incompatible with the concept that the internal differentiation of heterochromatin is less than that of euchromatin.

7) Views concerning the location of polygenes are similarly open to question.

8) The occurrence of a much smaller neo-Y in *T. destructor* and its failure to show heteropycnosis suggests that the neo-Y in *confusum* is in large part inert.

9) The precise conjugation of the X and Y pairing arms proves them to be homologous and proves, moreover, that heterochromatin is derivable from euchromatin.

10) In agreement with MULLER, the negative heteropycnosis and inertness of the Y are considered a response to its permanent isolation in the male sex.

11) If the centric blocks are also inert, their retention may be obligatory in so far as their loss would lower the chiasma frequency, already close to a minimum value, and hence the recombination index.

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